CONTROL OF LIVER CELL REPLICATION BY ALBUMIN NEED

ALAN D. SUDWEEKS and ROLLA B. HILL, JR. From the Department of Pathology, the University of Colorado School of Medicine, Denver, Colorado 80220

INTRODUCTION

Interest in relating biochemical differentiation to cytological events in various systems has recently been high. We wished to know whether the adult liver can be counted among those tissues in which onset of induced or increased differentiated function must be preceded by a period of DNA synthesis and cell division. More specifically, we have asked whether a need for increased synthesis of albumin by the liver might be a stimulus to cell division.

METHODS

Adult female white rats, weighing 200–250 g, were used. A pair of rats, matched for weight, constituted the control and experimental rats for one experiment. At 9:00 a.m., ether anesthesia was induced; an external jugular vein was cannulated in each rat; the rats were heparinized; and one-third of the calculated circulating blood volume, calculated as 50 ml/kg, was withdrawn. All operations were performed simultaneously in the two rats. In the control rat, the whole blood was returned immediately; in the experimental rat, it was replaced immediately with an equal volume of fresh rat erythrocytes suspended in saline with 10% glucose at a hematocrit equal to that of whole blood. This procedure was repeated, in both rats, at half-hourly intervals. 5 min after the third plasmapheresis, ½ ml of blood was removed for protein determination by cellulose-acetate electrophoresis. The veins were tied off, both cannulae were removed, and the incisions were closed with clips. Tritiated thymidine, 1 μc/g body weight, then was given intraperitoneally to each rat at 16, 19, and 22 hr after plasmapheresis. At 48 hr after plasmapheresis both the experimental and control animals were sacrificed. Samples from all four major lobes of the liver of each rat were sectioned and radioautographed. A section of kidney from each rat was also examined.

The resulting radioautographs were scored as follows. In each section, 10 squares, 1 × 1 mm each, were selected by throw of dice. With the aid of a grid-marked slide, all labeled nuclei in these squares were counted. Thus, 40 mm^2 from each liver were scored for the presence of labeled nuclei.

RESULTS

The results are presented in Table I. Each animal suffered a considerable decrease in circulating albumin as a result of plasmapheresis. In livers of animals that had sham operation the labeling of nuclei was slightly increased over baseline levels, whereas in the livers of experimental animals the labeled nuclei showed a severalfold increase. Representative photomicrographs are seen in Fig. 1. Labeled nuclei were uncommon in kidney, and no increase in labeled nuclei was observed in kidney after plasmapheresis.

DISCUSSION

Glinos (1) has reported experiments that differ considerably in design from the present ones. Instead of utilizing thymidine incorporation and counting labeled nuclei, Glinos counted the number of mitoses at certain periods after plasmapheresis. The present results are consistent with those reported by Glinos.

The extent of the drop in circulating albumin is only suggested by the measured decrease in albumin concentration, since albumin concentration in the circulating compartment is rapidly restored by a decrease in blood volume and by an inflow of albumin from the extravascular compartment. Such a drop in plasma albumin may be confidently expected to be followed, within 2–3 days, by a marked (two- to threefold) increase in albumin synthesis by the liver (2) . One may infer from these experiments that, during the process of preparing to increase the rate of albumin production, the liver cells must synthesize DNA and divide. There are many examples from other systems, including collagen-producing cells (3), cells of the lens (4), mammary gland (5), bone marrow (6), spleen (7), antibody-producing cells (8), chondrocytes (9), diapausing tissues of the silkworm pupa (10), various epithelia (11), and differentiating kidney (12), and pancreas (13), which provide evidence that in other tissues as well a necessary first step in the production of a differentiated product is DNA synthesis and cell division. Evidence that this may be a general biological phenomenon is provided by experiments the results of which show that differentiated, nondividing cells are not susceptible to transformation by RNA viruses (14–15) and that prior to infection and

TABLE I

Nuclear Labeling and Plasma Albumin in Livers of Control and Plasmapheresed Rats

Labeled liver nuclei/mm²			
Control			
No bleeding	Bled*	Plasmapheresed*	Plasma albumin pre/post††
			g/100 ml
2 ± 1	9 ± 3	39 ± 18	2.96/1.24
2 ± 1	19 ± 6	80 ± 23	3.66/1.85
	2 ± 1	12 ± 8	3.95/2.04
	14 ± 4	34 ± 12	4.00/—
	8 ± 4	82 ± 21	3.25/2.04
	12 ± 6	25 ± 11	3.47/2.50
		50 ± 16	3.90/2.64
		60 ± 17	4.07/2.40

* The experiments on the control, bled, and the plasmapheresed animals whose labeled liver nuclei are reported on the same line were performed simultaneously.

†† These data refer to the plasma albumin in the blood of the experimental rats, before and after plasmapheresis.

transformation DNA synthesis must occur in the host cell (16–18).

It appears possible that rearrangements of chromosomal material may occur during DNA synthesis and mitosis, which result in a change in differentiated phenotype of the cells. Possibly this change in differentiation cannot occur without such rearrangements. In the case of the experiments reported herein, we suggest the hypothesis that cells previously dedicated to other functions[1] were diverted, by means of such rearrangements, to albumin production, in response to the marked lowering of total body albumin.

A corollary hypothesis suggested by these ex-

[1] Y. Hamashima, J. G. Harter, and A. H. Coons (1964. *J. Cell Biol.* **20**:271) and M. I. Barnhart (1960. *Am. J. Physiol.* **199**:360) have reported evidence which indicates a distribution of differentiated functions among hepatocytes. M. G. Cunning and P. E. Hughes (1964. *Exptl. Cell Res.* **36**:592) report experiments which suggest that only certain specific hepatocytes are capable of division in the early response to a regenerative stimulus.

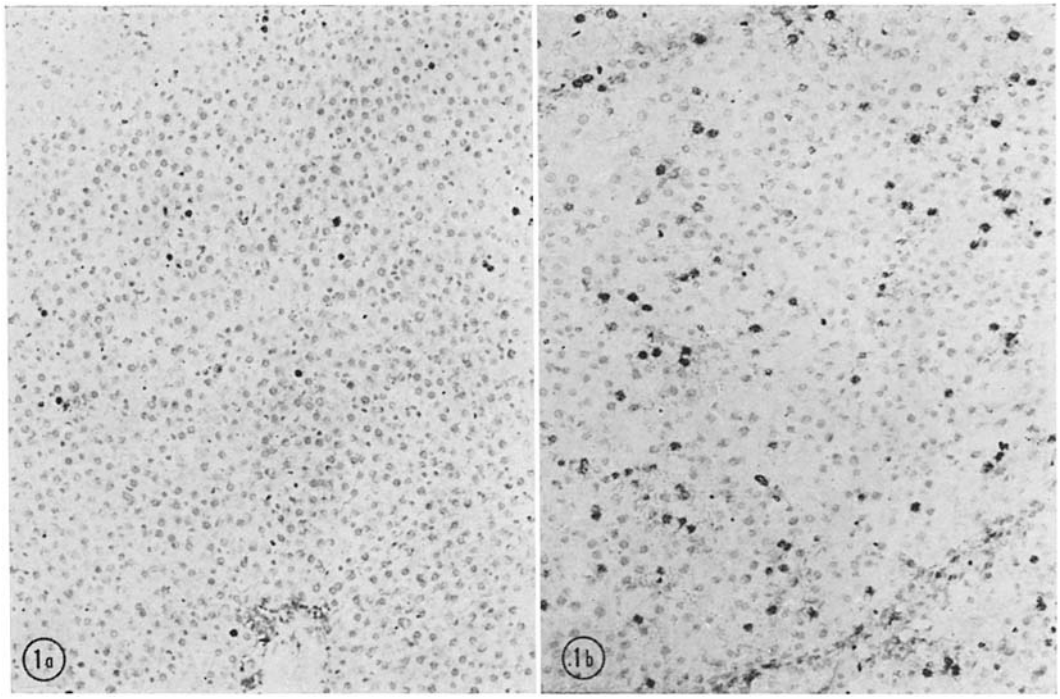

FIGURE 1 Photomicrographs of radioautograms of liver. Each photo includes two portal zones and the lobular tissue between. (*a*) Control, bled animal; relatively few labeled nuclei are seen. (*b*) Plasmapheresed animals; labeled nuclei are present in large numbers. × 1000.

periments is that major variations in albumin synthesis cannot be achieved through ordinary intracellular controls, which presumably operate over a much narrower range. The need for a large increase in albumin output requires that the number of albumin-producing cells be increased; conversely, a large decrease in albumin production can only come about through a decrease in the number of albumin-producing cells. Support for this hypothesis comes from experiments in which the total body albumin is greatly increased by the injection of albumin; in this situation albumin catabolism is proportionately increased, but hepatic synthesis of albumin appears to continue at approximately its normal rate for up to 2 wk.[2]

[2] Franks, J. J. Data in preparation.

SUMMARY

Plasmapheresis in the rat is followed promptly by a sharp increase of DNA synthesis in liver parenchymal cells. It is suggested that the rate of production of differentiated products of the liver, e.g. albumin, is controlled largely by the number of synthesizing cells, and that in order to increase markedly the hepatic synthesis of albumin DNA synthesis and cell division must first take place. In addition, a hypothesis is proposed that distribution of functions among hepatocytes may be partially controlled during DNA synthesis or mitosis.

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