TABLE I. Transformation of 24-C^{14}-Labeled Cholic, Deoxycholic and Chenodeoxycholic Acids in Anaerobic Subcultures of Mixed Human Fecal Microorganisms.

Serial subculture from feces	Bile acid .2 mM/l Trypticase soy broth	pH of broth after 3 days incubation	% distribution of isotope in Supernatant broth	Phosphate buffer washing	Acetone extract of sediment	Labeled metabolities without hydroxyl or keto group at C-7, as % of total amt of labeled material present in: Supernatant	Sediment
1	Chenode-oxycholate	7.2	35	2	63	0	98
2	"	6.9	48	2	50	4	96
3	"		49	4	47	8	98
4	"		44	2	54	8	97
5	"	6.8	48	2	50	9	97
6	"	6.8	46	2	52	9	97
7	"	7.2	46	2	52	10	95
7	Cholate	7.2	97	1	2	70	—
7	Deoxycholate	7.2	96	2	2	—	—

high ability for removal of the 7-hydroxyl group from cholic as well as from chenodeoxycholic acid. Centrifugation of the subcultures at 25,000 × *g* for 60 minutes reveals that the 2 reaction products appear in a different physical state. Deoxycholic acid appears in the supernatant whereas lithocholic acid is recovered mainly in an acetone extract of the sediment. These results indicate that lithocholic acid formed *in vivo* in the human intestine probably is less accessible for absorption than deoxycholic acid and therefore is prevented from entering the enterohepatic circulation. This might also explain why lithocholic acid is found only in trace amounts in the human bile.

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Cell Division and Phagocytic Activity in Liver Reticulo-Endothelial Cells. (27578)

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Evidence has been presented that stimulation to hyperactivity of the reticulo-endothelial (R. E.) system by estradiol or zymosan and recovery of the R. E. system from "blockade" by colloids are both accompanied by liver littoral* cell proliferation(1). A

* The term littoral cell rather than reticulo-endothelial cell has been used here after Abercrombie and Harkness(2), because some uncertainty has existed as to whether or not all the sinusoidal lining cells are capable of phagocytosis and thus whether or not they belong to the reticulo-endothelial system.

TABLE I.

Experiment	% liver littoral cells labeled with H^3-thymidine: With colloid, Centrilobular	With colloid, Perilobular	Without colloid, Centrilobular	Without colloid, Perilobular	% parenchymal cells labeled H^3-thymidine, Centrilobular	Parenchymal, Perilobular	% littoral cells with colloid, Centrilobular	Littoral, Perilobular
(1a) Phagocytosis by stimulated cells during DNA synthetic period (3 day estradiol; 3 hr H^3-thymidine; 2 hr colloid)	11.6	11.8	.0	.0	3.3	.0	64	59
	9.3	9.3	.0	.0	.9	.6	56	64
	6.7	11.4	.0	.0	.5	1.3	56	65
Avg	9.2	10.8	.0	.0	1.6	.6	59	63
(1b) Phagocytosis by newly divided stimulated cells (4 day estradiol; 1 day H^3-thymidine; 2 hr colloid)	10.9	16.0	.0	.4	1.4	.0	58	64
	11.1	13.4	.0	.0	2.7	.0	57	61
	10.4	12.8	.0	.0	1.0	1.2	42	52
Avg	10.8	14.1	.0	.1	1.7	.4	52	59
(2) Stimulation of proliferation by colloid loading (2 day colloid; 1 hr H^3-thymidine)	8.7	5.3	1.5	.6	2.3	5.4	49	44
	5.1	2.6	.0	.2	1.9	3.3	49	49
	9.1	5.4	1.4	1.2	2.8	1.2	46	45
	10.3	5.0	2.1	1.1	.6	5.2	42	54
Avg	8.3	4.6	1.2	.8	1.9	3.8	46	48
Unstimulated controls (n=8) (H^3-thymidine only) Avg ± S.E.		1.5 ± .4			.02 ± .13		—	

question has frequently been raised as to which littoral cells undergo division under these conditions. Are the active phagocytes in the liver the cells which divide or is there a group of less differentiated stem cells which supplies the highly active phagocytic population? The experiments reported here were designed to elucidate these points under conditions which have previously been shown to stimulate liver littoral cell proliferation: estradiol administration and recovery from "blockade" by saccharated iron oxide.

Methods. Swiss male mice approximately 8 weeks old were used in all experiments. In Exp. 1, liver littoral cell division was stimulated by subcutaneous administration of 1 mg of estradiol as previously described(1). Three days later tritiated thymidine (25 μc per mouse, 360 μc/μmole, Schwartz Laboratories) was injected intraperitoneally to label the cells synthesizing DNA in preparation for division. To determine whether cells preparing for division were phagocytic, saccharated iron oxide (Proferrin, Sharp and Dohme) containing 1 mg iron was injected intravenously one hour after the thymidine, and the mice were sacrificed 2 hours later (Exp. 1a). In another group (Exp. 1b) the saccharated iron oxide was injected one day after the thymidine, and the mice were sacrificed 2 hours later to determine whether newly divided cells were phagocytic.

In Exp. 2, the same dose of saccharated iron oxide was employed for a different purpose, *i.e.*, as a "blockading" agent. To determine which cells were preparing for division in the recovery from "blockade", tritiated thymidine was given 2 days after intravenous injection of the "blockading" dose of colloid and the mice were sacrificed 1 hour later.

Following paraffin embedding: 6 μ liver sections were covered with stripping film (Kodak AR10), exposed for 3 weeks, developed and stained with hematoxylin and eosin. In each liver approximatley 1000 cells were examined at a magnification of 900× in both the perilobular regions and in the areas closely surrounding central veins. The cells were scored as to whether or not they contained saccharated iron oxide and whether or not they had incorporated labeled thymidine into their DNA.

In order to demonstrate more clearly phagocytized colloidal iron, a duplicate set of sections was stained with Perls' ferrocyanide method and counter stained with hematoxylin to make the nuclei clearly visible. In each liver approximately 400 cells were scored at

a magnification of 900× in the perilobular regions and an equal number in areas closely surrounding central veins.

Results and discussion. The data are presented in Table I. Each entry represents the scoring on one liver. From the data of Exp. 1 it is clear that DNA synthesis is markedly increased in both littoral and parenchymal cells following administration of estradiol. The littoral cells which have been stimulated are capable of phagocytizing colloid during the DNA synthetic period (Exp. 1a) and shortly after they presumably have divided (Exp. 1b).

From 10 to 15% of the liver littoral cells were labeled with tritiated thymidine in the estradiol treated mice, and all of these labeled cells also contained colloid. However, phagocytized colloid was observable in only about half (50-60%) of the total littoral cell population in these livers. Had labeled and nonlabeled cells possessed equal phagocytic potential, we would have expected only half of the labeled cells to contain colloid. These observations suggest that the cells which have been stimulated to divide by estradiol treatment are particularly avid phagocytes.

Exp. 2 was designed to test whether colloid loaded cells are capable of division or whether the recovery from "blockade" is a result of proliferation of unloaded cells or "stem cells". Since most of the thymidine labeled littoral cells contained colloid, we must conclude that the active phagocytes are capable of DNA synthesis after ingesting colloid, and that to a large extent, the littoral cells which are preparing for division come from the colloid containing population.

In this experiment it is noted that some of the cells which do not appear to contain colloid are also preparing for division. This observation, though suggestive, cannot establish with certainty that the labeled cells without observable colloid were stimulated by some intercellular mechanism. There are 3 alternative explanations for this observation: these cells may have contained colloid which was not recognized; the labeled cells without colloid could have resulted from a colloid shedding division during the 2-day period before the tritiated thymidine was injected; they may have been in the normal pool of dividing cells and did not happen to phagocytize at the time the colloid was available.

Although cell division normally follows DNA synthesis, it seemed possible that those cells which contained colloid might fail to enter the mitotic division for which they had prepared. Therefore, sections were scanned for mitotic figures. About 0.5 to 1% of the littoral cells were found to be in mitosis. Colloid was visible in approximately 80% of these cells so that the fraction of dividing cells containing colloid was similar to the fraction of DNA synthesizing cells containing colloid. This indicates that the ingested colloid did not interfere with the division process to any major extent, and under *in vivo* conditions, confirms the observation made many years ago by Beard and Rous(3) that Kupffer cells which had ingested colloidal material were capable of division in culture.

We have previously reported(1) a very high liver DNA specific activity 2 days after intravenous injection of saccharated iron oxide. In the present experiment the radioautographic data (Exp. 2, Table I) indicate that this DNA synthesis is not confined to the littoral cells in the colloid-loaded livers. The large increase which occurred in the percentage of parenchymal cells synthesizing DNA and undergoing mitosis, together with their relative abundance and polyploidy, makes their contribution a sizeable fraction of the total liver DNA synthesis. The explanation for the parenchymal cell proliferation is unknown but may be a response to mild injury.

The fraction of littoral cells containing colloid was found to be the same in perilobular and centrilobular regions in sections which had been prepared primarily for radioautography. Howard *et al.*(4) had, however, reported marked perilobular distribution of colloidal iron. Duplicate sections were therefore reexamined after staining with Perls' stain for ferric iron in order to make the colloid more clearly visible. When these sections were examined under low magnification, it was obvious that most of the iron was located in perilobular regions confirming the observation of Howard *et al.* Scoring under high power,

TABLE II.

Iron distribution (Perls' stain) and cell populations in livers	% littoral cells with colloid		Littoral cell nuclei per 100 parenchymal cell nuclei	
	Centri-lobular	Peri-lobular	Centri-lobular	Peri-lobular
(1a) Mice given estradiol 3 days, and colloid 2 hr before sacrifice	65	74	110	160
	68	64	76	103
	80	88	131	175
Avg	71	75	106	146
(1b) Mice given estradiol 4 days, and colloid 2 hr before sacrifice	62	68	96	141
	70	74	116	170
	67	76	104	184
Avg	66	73	105	165
(2) Mice given colloid 2 days before sacrifice	52	61	118	115
	49	45	90	106
	38	46	87	132
	42	49	95	116
Avg	45	50	98	117
(Controls) Mice given colloid 2 hr before sacrifice	74	79	76	101
	57	60	66	95
	50	61	66	106
Avg	60	67	69	101

however, again showed no essential difference in the percentage of littoral cells containing colloid in the 2 regions (Table II). Two factors appear to account for the observation that the perilobular areas contain a large proportion of the total iron. The individual phagocytes in this region generally contain a larger amount of colloid per cell than do the centrilobular phagocytes, and concentration of littoral cells is higher in the perilobular region, as indicated by the ratio of littoral cell nuclei to parenchymal cell nuclei (Table II).

The overall percentage of littoral cells containing recognizable colloid was slightly higher in sections stained with Perls' stain (Table II) than in the sections used for radioautographs (Table I). This is probably due to the fact that the unstained colloid was more difficult to see and some cells containing small amounts of colloid were therefore scored as negative.

The livers of mice stimulated with estradiol had a higher ratio of littoral cell to parenchymal cell nuclei than the controls (Table II), confirming this point in our previous report(1). Both the relative increase in littoral cell number (Table II) and the percent labeling (Table I) were similar in the perilobular and centrilobular regions, indicating that cells in both regions proliferated equally. In contrast, in the animals recovering from "blockade" the thymidine labeling was always higher in the central areas than in the perilobular areas (Table I, Exp. 2). Bensch *et al.* (5) pointed out that fibroblasts which have ingested small amounts of particulate material divide normally, but that cells which have ingested very large amounts do not divide. It may be that the perilobular phagocytes, which have ingested a great deal more colloid than the centrilobular phagocytes, are to some extent prevented from undergoing DNA synthesis and division. This may also explain the fact that the relative increase over control values in cell number (littoral cell nuclei/100 parenchymal cell nuclei) is greater in the central than in the perilobular areas in the colloid-loaded livers (Table II).

It is important to note that the experiments reported here relate only to the proliferation of the littoral cells of the liver and that this is but one of the possible mechanisms of R. E. system activation(6). Thus, following endotoxin injection, Howard(7) has described conversion of "F" type to "G" type littoral cells(8), and an increase in acid phosphatase content of existing liver littoral cells.

Summary. Radioautographs of liver sections from mice given tritiated thymidine

were used to identify the cells which were preparing for division and colloidal saccharated iron oxide was used to identify the active phagocytic cells. In livers of mice whose reticulo-endothelial system was stimulated by estradiol, it was established that the cells preparing for division and those which had recently divided were actively phagocytic. In livers of mice whose reticulo-endothelial system had been "blockaded" with saccharated iron oxide, it was established that the cells which had phagocytized colloid were able to divide in the process of recovery from "blockade." No evidence was found for a stem cell which proliferates and differentiates to provide the active phagocytic population.

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Origin of the Galvanic Skin Response.* (27579)

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If a metal plate electrode is placed on the skin surface of the human palm, or on the foot or toe pads of the cat, and the body of the experimental subject grounded by means of another metal plate electrode at some point on the body at a distance from the first electrode, a change in electrical potential will occur between the 2 electrodes in response to a stimulus transmitted to the skin by the sympathetic nervous system. There is a simultaneous decrease in the skin's resistance to the passage of an electric current. When the potential change is elicited by any painful, startling, or threatening event in the environment of the experimental subject, it is referred to as the galvanic skin *reflex*. When the stimulus is applied peripherally as when a peripheral sudomotor nerve, or the sympathetic trunk to the hind extremity is stimulated, the potential change is called the galvanic skin *response*. The wave forms of the galvanic skin reflex and the galvanic skin response, when recorded from metal plate electrodes (hereafter referred to as "macroelectrodes") covering a portion of the skin's surface, are identical. Their latent periods differ as would be expected from the difference in the path length and conduction time of the nerve fibers carrying the stimulus to the skin(1).

Various theories of origin of the galvanic skin reflex and the galvanic skin response have been advanced at one time or another since their discovery by Fere(2) and Tarchanoff(3), respectively, over 70 years ago. Veraguth(4), by implanting the grounded, or indifferent, electrode beneath the skin surface, demonstrated that the potential change originated within the skin, rather than from muscle and other underlying tissues. The controversy primarily centers around the question of whether this phenomenon is caused by sweating, the widely accepted view. These theories and the experimental evidence supporting them have been reviewed by Wang (1).

Richter(5) pointed out that the skin po-

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