22. Baker, D. E., Proc. Gnotoiobte Workshop and Symp., Ohio State, **1963**, 68.
23. Nodell, J. and Schwartz, S., Proc. Soc. Exptl. Biol. Med. **116**, 395 (1964).

Received Dec. 18, 1967. P.S.E.B.M., 1968, Vol. 128.

The Effect of Pregnancy on the Cytology of Mouse Peritoneal Fluid (32963)

LARRY MCGOWAN, ROBERT H. DAVIS, AND LESLIE S. KREISLER
Department of Obstetrics and Gynecology, Thomas M. Fitzgerald Mercy Hospital, Darby, Pennsylvania 19023; and Department of Obstetrics and Gynecology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102

Pregnancy profoundly influenced the composition of serous body cells in women by significantly lowering mesothelial cells and lymphocytes and elevating polymorphonuclear leukocytes when compared to nonpregnant and postpartum women (1–3). In order to make possible useful comparisons with women, the present study was undertaken to determine the effect of pregnancy on the cytology of mouse peritoneal fluid. To our knowledge, no similar studies have been reported.

Materials and Methods. We cohabited adult female mice (CF-1; 25–30 gm) with adult males (30 gm) of the same strain in a 3:1 ratio under standard animal room conditions. Every morning, we inspected the females for a vaginal plug to establish day 1 of pregnancy. The serous fluid from the abdominal cavity of pregnant females was aspirated by us on days 3, 9, and 17 of gestation as well as 1 week postpartum by means of a No. 27 gauge needle attached to a 1-ml tuberculin syringe. In order not to penetrate the viscera, we introduced the needle into the abdominal cavity with the animal's ventral surface facing upward. By holding the needle firmly in place, we rotated the mouse back to its normal position so that fluid would drain toward the needle. We spread the aspirated specimen on an albumin-coated slide and stained it by Papanicolaou's procedure (4). Two hundred consecutive cells were counted by us and were grouped as mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, mast cells, and "other cells". The last category was less then 1% of the total sample and contained nuclei without cytoplasm which could not be further identified. Abdominal fluid was aspirated from nonpregnant control mice on the same days as the pregnant females so that 60 normal control counts from 15 animals were averaged to obtain the mean normal control values.

The significance of difference between counts taken on different days of gestation was computed using the formula, $SE = [\Sigma d^2/N(N-1)]^{1/2}$ and Student's t test. We calculated the standard error for each mean cell count and the probability values (p). To characterize the cells of the mouse peritoneal cavity, standard morphologic criteria were used by us (4).

Results. The influence of each stage of pregnancy and 1 week postpartum on mouse peritoneal fluid cytodifferential counts is seen in Table I. In normal control cytologic aspirations, the percentage of mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, mast cells, and "other cells" were 56.0, 33.7, 3.2, 4.1, 0.4, and 2.6%, respectively.

The number of mesothelial cells were significantly low ($p = 0.02$) in cytologic specimens obtained on day 9 of gestation when compared to day 3. Lymphocytes decreased on day 17 ($p < 0.05$) when compared to day 9, but returned to normal in the postpartum period.

As pregnancy progressed, polymorphonuclear leukocytes increased in a stepwise fashion. The control polymorphonuclear leukocyte count was 6.5 ± 0.7 which rose to 12.5 ± 1.4 on day 17 of pregnancy ($p < 0.01$). This change in cell count represents a 92.3% increase in polymorphonuclear leu-

TABLE I. Effect of Pregnancy on Mouse Peritoneal Fluid Cytodifferential Counts.

Groups	No. of mice	No. of cells per 200 cells counted[a]					
		Mesothelial cells	Lymphocytes	Polymorphonuclear leukocytes	Histiocytes	Mast cells	Other cells
Normal control	15	112.0 ± 2.1	67.4 ± 2.2	6.5 ± 0.7	8.3 ± 0.6	0.6 ± 0.1	5.2 ± 0.9
Pregnancy							
Day 3	30	114.1 ± 3.8	62.8 ± 4.6	7.1 ± 0.6	6.6 ± 1.0	0.4 ± 0.1	8.8 ± 2.0
Day 9	29	102.6 ± 2.8	68.1 ± 1.9	9.7 ± 0.6	11.9 ± 0.7	1.2 ± 0.5	3.7 ± 1.0
Day 17	30	112.7 ± 3.2	55.1 ± 5.9	12.5 ± 1.4	11.1 ± 0.8	0.4 ± 0.1	8.2 ± 3.3
Postpartum 1 week	32	107.7 ± 3.6	64.5 ± 3.9	3.6 ± 0.7	10.2 ± 1.5	1.0 ± 0.3	11.7 ± 2.2

[a] ± = SE of mean.

kocytes as a result of pregnancy. The 1 week postpartum value of 3.6 ± 0.7 was lower than the normal control count of 6.5 ± 0.7 ($p<0.01$).

During the latter part of pregnancy, the number of histiocytes also increased ($p<0.01$), but we observed no significant effect on the number and frequency of mast cells. After delivery, "other cells" were noted to be somewhat higher.

Discussion. The results of the present study suggest that pregnancy modifies mouse peritoneal fluid cytology in a way similar to the changes observed in women. In normal control mice the relative cell proportions differed somewhat from counts made on asymptomatic women. The cytodifferential counts in women contained on the average 25% mesothelial cells, 25% lymphocytes, 23% erthrocytes, 15% polymorphonuclear leukocytes, 8% histiocytes, and 4% squamous cells. We never observed contaminants such as red blood cells or squamous cells in mouse specimens.

In pregnant mice, mesothelial cells decreased significantly on day 9 which corresponds to the reduction observed in pregnant women during the second trimester. Possibly, the rising levels of adrenal steroids and/or estrogens during pregnancy reduced the mesothelial cells.

In pregnant women, polymorphonuclear leukocytes increased from 17.3 ± 6.9 in the first trimester to 60.1 ± 10.7 in the third trimester which represents a 140% increase in cells as compared to a 92.3% increase in pregnant mice which resembles the progressive rise in hormone levels, particularly estrogen, during pregnancy.

Lymphocytes in pregnant women decreased in the second and third trimesters of pregnancy, and in mice the general reduction of lymphocytes was noted on day 17. We observed an inverse image between polymorphonuclear leukocytes and lymphocytes in both mice and women by comparing counts of normal controls, pregnant, and postpartum specimens.

Unlike the human, mouse histiocytes in the peritoneal fluid increased during later pregnancy. There was no significant alteration in mast cell counts in pregnant mice, and these cells were so rare in women, we did not count them.

Summary. We aspirated peritoneal fluid from pregnant mice on days 3, 9, and 17, as well as 1 week postpartum. A reduction of mesothelial cells was observed on day 9 whereas lymphocytes decreased on day 17 of gestation. Polymorphonuclear leukocytes steadily increased as pregnancy progressed and in general, presented an inverse image with lymphocytes. Pregnancy increased histiocytes, but we did not notice a significant change in mast cells. The results suggest that the hormones of pregnancy alter the peritoneal fluid cytology in mice and some of the changes are similar to those previously observed by us in women.

1. McGowan, L., Stein, D. B., and Miller, W., Am.

J. Obstet. Gynecol. 96, 413 (1966).

2. McGowan, L., Davis, R. H., Stein, D. B., Bebon, S., and Vaskelis, P., Obstet. Gynecol. 30, 821 (1967).

3. McGowan, L., Davis, R. H., Stein, D. B., Bebon, S. and Vaskelis, P., Am. J. Clin. Pathol., 1968, in press.

4. Papanicolaou, G. N., "Atlas of Exfoliative Cytology", Harvard Univ. Press, Cambridge, Massachusetts, 1963.

5. Helmy, I. D. and Nicol, T., J. Obstet. Gynaec. Brit. Commonwealth 58, 588 (1951).

Received Dec. 20, 1967. P.S.E.B.M., 1968, Vol. 128.

Impaired Secretion of Triglycerides by the Liver; A Cause of Tetracycline-Induced Fatty Liver* (32964)

CHRISTOPHER H. HANSEN, LALEAH H. PEARSON, STEVEN SCHENKER,[1] AND BURTON COMBES[2] (Introduced by J. D. Wilson)

Liver-Gastroenterology Unit, The Department of Internal Medicine, The University of Texas Southwestern Medical School at Dallas, Dallas, Texas 75235

Tetracycline in large doses induces a fatty liver in experimental animals and has been incriminated as a cause of fatty liver and hepatic dysfunction in man (1–4). Most of the experimentally induced rise in hepatic lipids has been accounted for by an increase in liver triglycerides (2), but the mechanism by which tetracycline induces a fatty liver is unknown.

Inasmuch as tetracyclines inhibit protein synthesis *in vitro* (5) and *in vivo* (6), and triglycerides are normally bound to hepatic lipid acceptor protein and are transported from liver to plasma as lipoproteins (7), it seemed possible that tetracycline, in high concentrations, might inhibit hepatic lipid acceptor protein synthesis and thus impair the normal removal of triglycerides from the liver. Such a mechanism has been implicated in other types of fatty liver (8,9). To test this hypothesis, tetracycline-injected and control fasted rats were given Triton, a nonionic detergent, which blocks the exit of triglycerides from the plasma compartment so that triglycerides secreted by the liver accumulate in the plasma (8,10). A decrease of post-Triton hypertriglyceridemia in fasted tetracycline–injected rats, when accompanied by a fatty liver, was taken as evidence of an impaired secretion of triglycerides into plasma.

Material and Methods. Female Sprague-Dawley rats, weighing 200–250 gm and fasted for 24 or 48 hours were used. Tetracycline hydrochloride (Achromycin), supplied in vials containing 500 mg of tetracycline and buffered with 1250 mg of ascorbic acid, was freshly dissolved in isotonic saline to a concentration of 10 mg of tetracycline/ml. This solution, at pH 2.45, was infused via a femoral vein in a dose of 100 mg of tetracycline/kg of body weight, at the rate of 50 mg/kg per min, into rats briefly maintained under ethyl ether. This dose of tetracycline has been shown previously (1,2) and in preliminary experiments in this study, to induce a fatty liver in rats. Control animals received a comparable weight-adjusted volume of isotonic saline without ascorbate (pH 6.10), or in five instances, containing ascorbate in an amount equivalent to that present in the tetracycline solution, and adjusted to pH 2.45 with dilute HCl. At various times thereafter (see Table I), both tetracycline-injected and control groups were infused with Triton, prepared as described previously (8), at a dose of 50 mg/100 gm of body weight. Plasma was obtained for triglyceride assay at 0.75–3 hours after Triton. Liver triglycerides were meas-

* This investigation was supported in part by Research Grants NB-05841 and HE 03439 from the United States Public Health Service.

[1] John and Mary R. Markle Scholar in Academic Medicine.

[2] Recipient of United States Public Health Service Research Career Program Award K3-AM 18,260 from the National Institute of Arthritis and Metabolic Diseases.