Effect of Birth on Fetal Mouse Peritoneal Fluid Cellular Composition (37149)

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Previous studies in our laboratory have shown that many important physiologic factors characteristically influence the cellular content of abdominal fluid. For example, advancing pregnancy in mice (1) and women (2) gradually increased the proportion of polymorphonuclear leukocytes and decreased the relative amounts of lymphocytes in cytologic aspirates. Lymphocyte distributions increased in peritoneal fluid with advancing age from birth, however, mast cells were lower in mature animals than day-old mice (3). In order to better understand the influence of birth on peritoneal fluid cytology the peritoneal fluid % cellular distributions of 18-day-old fetuses were compared with day-old newborn mice.

Materials and Methods. Adult female CF-1 mice (25–30 g) were cohabited with male animals of the same strain in a 3:1 ratio under standard animal room conditions. Every morning the females were inspected for vaginal plugs to establish Day 1 of pregnancy. On Day 18 of gestation fetuses were removed from the uterus and aspirated for abdominal fluid and on Day 1 postpartum newborn mice were also aspirated for peritoneal fluid. In order not to penetrate the viscera a 27 gauge needle attached to a 1 ml tuberculin syringe was introduced into the abdominal cavity with the animal's ventral surface facing upward. By holding the needle firmly in place the mouse was rotated back to its normal position so that fluid would drain into the needle. Care was taken not to stress the animal in obtaining fluid. The aspirated specimens were spread on albumin-coated slides, fixed in 95% alcohol and stained with histologic buffered Wright's stain. Two hundred consecutive cells were counted and grouped as lymphocytes, polymorphonuclear leukocytes, monocytes, eosinophils, other mononuclear cells and mast cells. This procedure of randomly counting a fixed number of cells rather than counting the number of cells in an absolute fluid volume eliminates the extreme variation resulting from counting cells per unit volume because of the small amount of fluid aspirated—sometimes only a drop or so. The standard errors of the various means were calculated using the formula, $SE = [\Sigma d^2/N(N-1)]^{1/2}$ and the p value using Student's t test. The coefficient of standard error variation, a rough index of experimental error, was obtained by dividing the standard error by the mean $\times$ 100 (4). Standard morphologic criteria was used to characterize the cells of the mouse peritoneal fluid cavity.

Results. Delivery and birth markedly influenced the cellular content of mouse fetal abdominal cavities. However, a causal relationship between delivery *per se* and peritoneal fluid content has not been established since there are a variety of changes which occur between Day 18 of gestation and postpartum Day 1. For example, maturation alone could account for some of the changes which may not be related to hormonal alteration. Considerably more fluid was obtained before birth from the abdominal cavities of 18-day-old fetuses than newborn mice. No attempt was made in this study to show the influence of fetal sex on fluid cell counts.

The % distribution of lymphocytes was 59.2 ± 2.5 in the 18-day-old fetus which increased to 68.2 ± 3.4 ($p < 0.05$) in the newborn. A twofold increase in peritoneal fluid polymorphonuclear leukocytes was also

recorded ($p < 0.001$) and more mast cells were seen in the newborn peritoneal aspirates. However, less eosinophil and other mononuclear cell proportions ($p < 0.001$) were noted after birth even though monocytes remained unchanged ($p > 0.5$ (Table I). The mean coefficient of standard error variation for this study is 19.0%.

Discussion. Pregnancy, age and sex differences have a profound influence on the distribution of cells within the abdominal cavity. In order to provide a new approach to problems of obstetrics and gynecology, we have shown that the cellular patterns are influenced by endocrine alterations, the estrous and menstrual cycles, radiation and inflammation (5). Peritoneal fluid cells are an excellent index of the internal milieu of the pelvis. In mice (1) and women (2) polymorphonuclear leukocytes increased in pregnancy which corresponds to the overall leukocytosis in blood. The low % distribution of polymorphonuclear leukocytes seen in the Day 18 fetal mouse may reflect the status of peripheral blood. Lymphocytes were significantly lower in pregnant than nonpregnant or postpartum aspirates. Possibly lymphocytes are shifting from the peripheral blood to peritoneal fluid of the fetus giving us some idea of how cells originate within the pelvic cavity. However, ratios of the various blood elements in Day 18 fetuses must be compared to the newborn. Certain animals depleted of lymphocytes can synthesize antibodies through some cell-bound immune mechanisms and tissue grafts have a prolonged survival (6). The change in peritoneal fluid cell distribution seen in the present study could reflect the immunologic relationship between fetus, mother and placenta. Certainly, the mouse provides the best model for mimicking women in pregnancy because the equilibrium with respect to the formation of new cells and the destruction of old ones probably is altered by pregnancy in a similar manner in both species. The cellular changes seen in birth point to many regulatory mechanisms so essential for delivery and birth as well as the attainment of full maturity. When one compares the lymphocyte counts of both sexes of mice one notices that lymphocytes increase with age even before birth (3). Lymphocytes probably serve a protective immune function coming probably from the thymus. Our data supports the concept that neonatal thymectomy produces lymphocytopenia, lymphoid tissue atrophy and impaired immune mechanisms needed for delivery and birth. There are many conflicting opinions on the responsiveness of the pituitary–adrenal system in the fetus and neonate. It is felt that the stress of delivery produces a hyperfunction of this system in the fetus and mother producing an excess glucocorticoids causing lymphoid elements to decrease in the mother. Mast cells become prominent at birth and possibly its components may be involved in delivery.

TABLE I. Effect of Birth on Fetal Mouse Peritoneal Fluid Cellular Content.

Cells	Age (days)		
	Fetus (Day 18 of pregnancy)	Newborn (day 1)	p value
No. of mice	15	12	—
Body wt (g)	0.38 ± 0.07	1.43 ± 0.20	—
	% distribution of cells per 200 cells counted		
Lymphocyte	59.2 ± 2.5[a]	68.2 ± 3.4	<0.05
Polymorphonuclear leukocytes	9.2 ± 0.9	20.9 ± 2.1	<0.001
Monocyte	1.9 ± 0.7	2.2 ± 0.5	>0.5
Eosinophil	1.4 ± 0.7	0.5 ± —	—
Other mononuclear cells	26.0 ± 2.9	6.6 ± 1.4	<0.001
Mast cells	0.3 ± —	2.1 ± 0.6	—

[a] ± Standard error of the mean.

Summary. Peritoneal fluid was aspirated from 18-day-old fetuses as well as Day 1 newborn CF-1 mice and was placed on an albumin-coated slide, fixed in 95% alcohol and stained by histologic buffered Wright's stain. The % distribution of lymphocytes, polymorphonuclear leukocytes and mast cells increased in mouse fetuses possibly as the result of delivery and birth but no causal relationship was established. More fluid was obtained before birth than after in the newborn. Other mononuclear cells and

eosinophils were less in peritoneal fluid of newborn mice.

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