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Effect on Blood Picture of 15-Day Embryo of Liver Extract Injected into Pregnant Rat.*

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The morphological similarity of primitive, pre-hepatic erythropoiesis in the embryo and megaloblastic erythropoiesis in untreated pernicious anemia[1, 2] has prompted further study of the embryo with the view of developing a method of bioassay for the anti-anemia principle in liver extract. A relative deficiency of the anti-anemia principle may be the etiological agent in both types of erythropoiesis.[3] Extensive studies have been made on the response of newborn rats whose mothers have been treated with antianemia substances. Accelerated maturation has been reported[4–7] but has not been confirmed.[8, 9, 10] Recent observations of accelerated maturation of erythropoietic cells in the yolk sac of the 11-day rat fetus subsequent to liver and Ventriculin therapy in the pregnant rat emphasize the necessity of studying the prehepatic or "megaloblastic" generation of cells.[11, 12]

The circulating blood in the 15-day fetus is comprised predominantly of erythroblasts, normoblasts, and non-nucleated reticulocytes and erythrocytes of the prehepatic generation. Therefore, accelerated maturation of erythropoiesis in the 11-day fetus should appear in a study of the blood of the 15-day fetus. Since the per-

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centage of non-nucleated cells rises rapidly between the 14th and 18th days of fetal life, a significant increase above the control percentage value at 15 days represents accelerated maturation of primitive erythropoiesis.

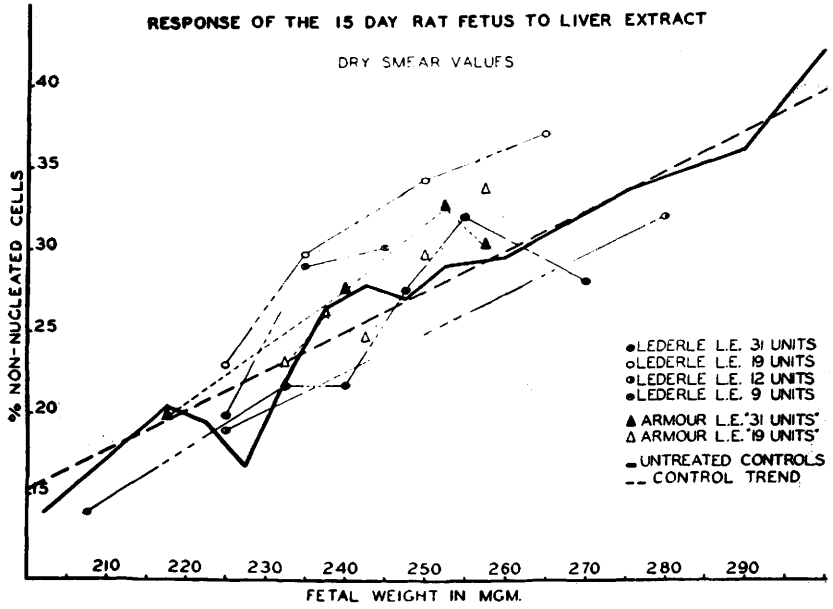
Methods and Technics. Young adult female rats were mated and on the 15th day of pregnancy the fetuses were removed under ether anesthesia with the amnion, yolk sac, and placental button intact. Fetal blood was obtained by puncturing the vitelline vessels. One sample of blood was smeared and stained with May-Grunwald-Giemsa stain. Supravitally stained wet preparations were also made by mixing another drop of blood with .18% brilliant cresyl blue in isotonic salt solution. 1000 cells per fetus were counted, 500 on the wet preparation and 500 on the dry smear. The choice of 4 fetuses per litter yielded a representative sample of the litter. The weight of each fetus was recorded, since the body weight was considered the most accurate gross measure of fetal development in the rat.[13] The rats were injected subcutaneously with 2 liver preparations at 4 dosage levels. The Lederle† liver extract contained 15 units per cc, whereas the Armour product‡ was assigned an arbitrary unitage upon the weight of fresh beef liver represented in each cc of extract. The animals were usually given 3.5 to 7.5 units on the sixth day of pregnancy and maintained on 1.5 u daily through the 14th day. Rats on the 31 u level received 7.5 u on days 6, 7, and 8, with subsequent 1.5 u maintenance dose daily. Thus the pregnant rats received liver therapy before, during and after the anlage of the mesoderm which gives rise to the blood-forming tissues in the fetus.

Results. Fig. 1 illustrates the percent non-nucleated cell values as plotted against fetal weight. Is the response of the animals receiving liver therapy biologically significant? The distribution of variation of the control animals contains 2 components, *i. e.*, the variation at any given fetal weight and the upward trend. Since the trend factor is fortuitous and unnecessary for comparison of a treated and untreated animal at any given fetal weight, it can be eliminated. By fitting a straight line to the control curve by the least squares method and by calculating the distribution of variation of control values from the straight line, the standard deviation can be determined independently of the trend. The means of percent counts on treated and untreated animals as compared with the

[13] Donaldson, H. H., *The Rat*, 1924.

† Generously contributed by Dr. Guy Clark of Lederle Laboratories.

‡ Obtained through the kindness of Armour Laboratories.



means of the calculated normal response determined from the straight line equation are listed in Table I. In no instance does a mean of a treated group of rats exceed the calculated control value by the conventional 2 sigma.

Summary. 1. Potent and standardized liver preparations were

TABLE I.
Comparison of Differential Counts on Treated and Control Fifteen-day Rat Embryos.

Therapy	Litters	Fetuses	Mean fetal wt in mg	% non-nucleated cells (mean values) Wet smears Expt.	Wet smears Calc.*	Dry smears Expt.	Dry smears Calc.*
Controls (untreated)	30	120	240	22.4	22.4	26.2	26.3
				$\sigma = 3.3$		$\sigma = 4.4$	
Lederle Liver Extr.							
A. 9 units	3	12	240	19.6	22.2	18.7	25.8
B. 12 "	3	12	240	20.1	20.7	26.4	23.8
C. 19 "	6	24	240	25.9	22.1	30.0	25.4
D. 31 "	9	36	240	21.8	22.6	25.3	26.5
Armour Liver Extr.							
A. "19 units"†	8	32	240	26.4	22.6	26.5	25.4
B. 31 " †	7	28	250	29.9	24.6	32.0	28.6

*Means of values calculated from straight line equation fitted to control data by the least squares method where X = fetal weight and Y = % non-nucleated cells.
†A unit equivalent to fifteen g of fresh beef liver.

injected into normal pregnant rats before, during and after the anlage of the mesoderm in the developing embryo. 2. Differential counts were made on wet and dry smears of blood from 15-day fetuses and the percent of non-nucleated red cells present taken as an index of maturation of the blood picture. 3. In a series of 264 treated and untreated fetuses from 66 litters of rats, statistical analysis of the data indicates the response in liver-treated animals to be within the range of biological variation.

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Effect of Progesterone Anesthesia on Systemic Blood Pressure.*

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It has recently been shown by Selye[1] that steroid hormones may exert an anesthetic effect. Since it is known that anesthetics in general tend to depress the blood pressure, it appeared of interest to determine the blood pressure effect of progesterone anesthesia as compared to that of one of the standard anesthetics.

Eight female adult albino rats of an average body weight of 150 g (142 to 155 g) were given sufficient progesterone in an oversaturated solution (100 mg per cc) intraperitoneally to induce full surgical anesthesia, and the blood pressure was determined at 10-minute intervals throughout the anesthesia until recovery was complete. The same animals were similarly followed with nembutal anesthesia 24 hours later. Blood pressure recordings were made indirectly on the tail with an apparatus for non-anesthetized animals.[2] The total dose of progesterone ranged from 17 to 22 mg. Such a range was necessary since some of the animals are more resistant to this preparation than others. The total dose of nembutal (pentobarbital sodium) was 1/10 cc of a commercial preparation (Abbott) which contained one grain per cc.

Table I presents the data for systolic blood pressures obtained

* The expenses of this investigation were defrayed by a grant received from the Schering Corporation of Bloomfield, N. J., through the courtesy of Doctors G. Stragnell and E. Schwenk who have also kindly supplied the progesterone used in these experiments.

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