Mortality of Chick Embryos upon Injection of Homologous Adult Cells.* (24727)

PAUL I. TERASAKI, JACK A. CANNON AND WILLIAM P. LONGMIRE, JR.
(Introduced by David T. Imagawa)
Dept. of Surgery, University of California Medical Center, Los Angeles

Injection of either adult chicken spleen cells or adult chicken leucocytes into chick embryos caused enlargement of spleens of embryos as well as hemolytic anemia and often death(1,2). Similar results were obtained in mice by Billingham and Brent(3,4) who found that newborn mice injected with spleen cells from adult mice were stunted in growth, possessed atrophied lymph nodes, and often died within 40-60 days after injection. These effects of homologous adult cells in chicks and mice have been interpreted by both groups of workers to be due to adult cells reacting immunologically against the embryonic host. During our studies on induction of tolerance with adult cells to homografts in the chicken, intravenous injection of some types of adult cells caused death. The mortality due to injection of embryonic blood, adult bone marrow, red blood cells, and white blood cells is reported here.

Methods. Various types of cells were obtained from adult chickens or embryos, treated in several ways, and injected intravenously into 14-16 day old chick embryos. Recipient chick embryos and donor chickens or embryos were always of different breeds, New Hampshire or White Leghorn. To obtain *white blood cells,* heparinized blood was centrifuged at 2,500 rpm (International Clinical Centrifuge) for 20 minutes and the buffy coat pipetted off with polyethylene tube (P.E. 260) attached to syringe, bent in half, placed in tube, and re-centrifuged at 4,400 rpm for 20 minutes. This procedure was repeated in a smaller bore polyethylene tubing (P.E. 100) to make final white blood cell suspension. White cells from 6 different donor chickens were tested in varying dosages. *Red blood cells* used for injection were obtained from the lower layer of first centrifugation of blood. *Bone marrow cells* were obtained by anesthetizing donor bird with intravenous injection of sodium pentothal, drilling 2 holes in the femur 3 to 5 cm apart, inserting tight-fitting 18 gauge hypodermic needles attached to two 20 ml syringes. The cells were then centrifuged at 2,500 rpm for 30 minutes, taken out, and recentrifuged in polyethylene tube at 4,500 rpm for 20 minutes. The upper pink colored layer was separated from the lower red layer and the cells suspended in saline for injection. *Embryonic blood* pooled from 26 embryos was taken from embryos incubated 14 or 16 days. An average of 0.7 and 1.0 ml of blood was obtained from 14 day embryos respectively. Upon centrifugation for 20 minutes at 2500 rpm the upper layer of white cells was taken up into a polyethylene tube and recentrifuged for 20 minutes at 4400 rpm. The resulting upper layer of cells and lower layer cells from the first centrifugation were then injected.

Results. White blood cells from adult chickens when injected intravenously into 14-16 day-old chick embryos caused the death of 42 of 52 embryos. Although 10 chicks hatched (19%), only 2 were still alive 2 weeks after hatching. Varying dosages from 600,000 to 106 million white blood cells had roughly similar effects on mortality (Table I). On examination of 36 of the dead embryos, the spleens were enlarged and often had necrotic spots (Fig. 1 and 2). Adult *red blood cells* did not cause as marked an effect on mortality as did adult white blood cells. Twenty-two of the 31 embryos (17%) hatched, and 15 lived at least 2 weeks after hatching. Of the dead embryos examined, 3 of 7 had enlarged spleens. *Bone marrow,* when divided into heavier and lighter centrifugal fractions, showed most of its activity in the lighter fraction. Thus, of chicks injected with cells from the upper layer, 33% hatched as compared to

* This investigation was supported by research grants from NIHPHS and Cancer Research Co-ordinating Comm., Univ. of Calif.

TABLE I. Viability of Chick Embryos following Injection of Various Homologous Cells.

No. of donors	Type of cells & No. of cells ($\times 10^6$) inj.	No. of eggs inj.	No. chicks hatched	No. chicks hatched & healthy	No. chicks alive at 2 wk	No. of chicks with enlarged spleens/No. of chicks examined
6	Adult white blood cells .6-106 WBC .05-16 RBC	52	10 (19%)	7 (13%)	2 (4%)	36/36
3	Adult red blood cells 32-235 RBC	31	22 (71%)	19 (61%)	15 (49%)	3/ 7
	Adult bone marrow:					
4	Upper centrifugal layer, .3-28 cells	48	16 (33%)	9 (19%)	6 (12%)	17/26
3	Lower centrifugal layer, 2.8-37 cells	33	23 (70%)	20 (60%)	14 (42%)	2/ 8
	Embryonic blood:					
26	Upper centrifugal layer, 3.7-14.3 cells	24	17 (71%)	12 (50%)	8 (33%)	0/ 7
26	Lower centrifugal layer, 6.7-275 cells	31	23 (74%)	20 (64%)	18 (58%)	0/10
3	Plasma—heparinized and citrated	30	30 (100%)	29 (96%)	28 (93%)	0/ 1
1	Serum	10	9 (90%)	8 (80%)	8 (80%)	0/ 1

70% injected with cells from the upper layer, 33% hatched as compared to 70% injected with cells from the lower layer; survival at 2 weeks was 12% as compared to 42%. Embryonic blood, pooled from 26 embryos and centrifuged, did not have a marked effect on rate of hatching. The upper centrifugal layer caused a greater mortality than the lower layer during the period after hatch: 33% lived to 2 weeks of age as compared to 58%. Injection of *plasma* or *serum* had no appreciable effect on mortality.

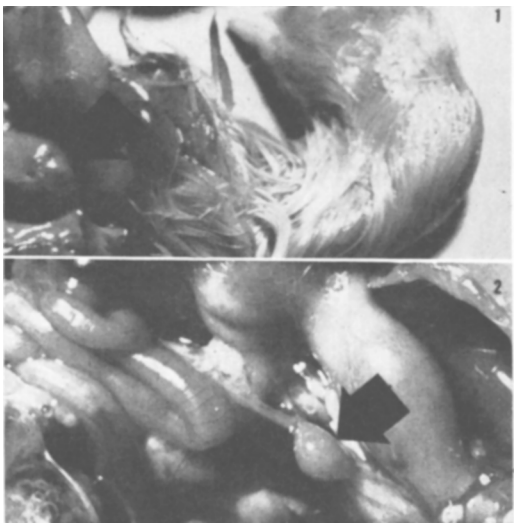

FIG. 1. A representative chick, inj. intrav. at 14 days incubation with white blood cells taken from adult chicken. Death occurred at 20 days incubation with enormous enlargement of spleen, indicated by arrow.

FIG. 2. Arrow indicates spleen of normal untreated chick on 21st day of incubation.

Discussion. Injection of certain types of adult chicken cells into 14-16 day chick embryos of another breed has been found to cause deaths of embryos, usually 5-7 days after injection. This degree of mortality was not obtained by injection of normal contaminants of cell preparations such as serum, heparinized plasma, or citrated plasma. Cells causing from 67-81% of deaths were adult circulating white cells and upper centrifugal layer of bone marrow cells. Invariably embryos injected with these cells die with greatly enlarged spleens. Cells causing 25-30% deaths at time of hatch were the adult red blood cells, lower centrifugal layer of bone marrow cells, and embryonic blood. This percentage of deaths could be considered to be slightly increased over normal experimental mortality since previous studies have shown that a 15-20% mortality could be expected after injection of blood from one embryo(5).

It seems fairly clear then that adult circulating white blood cells can produce death of embryos. Although bone marrow cells produced death, whether marrow cells free of

blood white cells can also be effective could not be determined due to the difficulty of obtaining marrow cells free of blood. The small amount of mortality and splenic enlargement due to injection of red blood cells could also be due to contamination with white blood cells.

The types of cells responsible for death seem to agree with the hypothesis of Billingham and Brent(3,4) and Simonsen(1,2) that such deaths are due to "graft *vs* host" reaction in which injected cells consider the embryo to be a homograft and thus react against it. Also consistent with this hypothesis is the fact that embryonic blood cells of the upper centrifugal layer caused delayed mortality when presumably they themselves had matured immunologically in the host.

The possibility that mortality and splenic enlargement is caused by infectious agents has been considered and ruled out by Billingham, Brent, and Simonsen(2,4).

Summary. Circulating white blood cells and the upper centrifugal layer of bone marrow cells taken from normal adult chickens caused a high percentage of deaths when injected into embryonic chicks. Considerably lower percentage of deaths was produced by injection of red blood cells, lower centrifugal layer of bone marrow cells, or embryonic blood.

The authors express appreciation for technical assistance of John McClelland and Mary Ann Coulson.

1. Simonsen, M., *Acta path. et microbiol. scandinav.*, 1957, v40, 480.

2. Cock, A. G., Simonsen, M., *Immunol.*, 1958, v1, 103.

3. Billingham, R. E., Brent, L., *Transplantation Bull.*, 1957, v4, 67.

4. ———, *Proc. Roy. Soc. B.*, 1958.

5. Terasaki, P., Cannon, J. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 103.

Received December 29, 1958. P.S.E.B.M., 1959, v100.

Effects of 2-Deoxyglucose on Blood Glucose Levels in the Rat.* (24728)

J. BROWN AND H. L. BACHRACH (Introduced by S. Roberts)
Dept. of Medicine, University of California at Los Angeles School of Medicine

Structural analogues of glucose have proved useful in elucidating some aspects of carbohydrate metabolism. Cramer and Woodward(1) found that 2-deoxyglucose inhibited yeast fermentation and glycolysis of Walker 256 carcinoma, apparently at a site prior to fructose-1, 6-diphosphate cleavage. Sols and Crane(2) observed that brain hexokinase converted 2-deoxyglycose to the 6-phosphate stage but this substance was not a substrate for phosphohexisomerase or glucose-6-phosphate dehydrogenase. Long and Thompson(3) confirmed these findings and suggested that 2-deoxyglucose inhibited hexokinase indirectly by causing accumulation of glucose-6-phosphate. Support for this conclusion was provided by Wick and co-workers(4) who found that 2-deoxyglucose acted as a competitive inhibitor of the phosphoglucoisomerase reaction. Administration of 150 mg/kg of 2-deoxyglucose to eviscerated rabbits reduced intracellular transfer rate of glucose to one-third during 8 hours with maximum insulin stimulation. Insulin accelerated movement of 2-deoxyglucose into rat diaphragms but glucose oxidation was substantially inhibited and fructose oxidation drastically reduced in this system. They proposed accumulation of glucose-6-phosphate with inhibition of hexokinase as the mechanism for inhibition of fructose utilization. The following studies were undertaken to determine the effects of 2-deoxyglucose on some aspects of carbohydrate metabolism in the intact rat.

Methods. Male Sprague-Dawley rats weighing 200-250 g were fasted overnight and anesthetized with sodium pentobarbital. Measure-

* This research was supported by USPHS grant (A-1847) from Nat. Inst. of Arthritis and Metabolic Diseases.