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.

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

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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-

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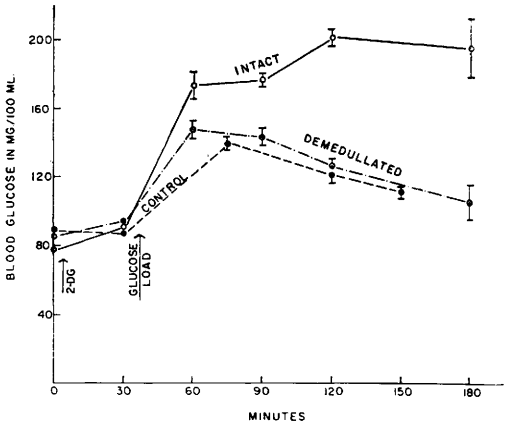

FIG. 1. Blood glucose response to intraper. glucose load (1500 mg/kg) given after 30 min. glucose determination. Effects of 2-deoxyglucose (400 mg/kg) intrav. on intact and demedullated groups compared to saline control.

ment of blood glucose was made in duplicate on 0.2 ml samples of tail blood using the Nelson modification of the Somogyi method(5). 2-deoxy-D-glucose was injected into the exposed saphenous vein in 10% solution 15-30 minutes before the glucose load which was injected intraperitoneally as a 7.5% solution. Injection of deoxyglucose in doses of 400 mg/kg raised blood glucose concentration as determined by this method, less than 10 mg/100 ml at 15-30 minutes after injection and in all cases a second blood glucose determination was made before the glucose load was injected. Adrenal demedullation was performed on 60-80 g male rats at least 4 weeks prior to use to permit cortical regeneration. Adrenals were examined at the end of experiment for completeness of adrenal demedullation. Dihydroergotamine was injected intravenously 15-20 minutes before deoxyglucose at a dose of 1 mg/kg.

Results. Initial experiments were designed to determine effects of injection of varying doses of 2-deoxyglucose on ability of groups of intact anesthetized rats to dispose of a glucose load. Fig. 1 (upper curve) shows results of intravenous injection of 400 mg/kg of deoxyglucose given after initial blood glucose determination, followed by 1500 mg/kg of glucose after second blood glucose determination. These results when compared with those from saline-treated glucose-loaded rats (lower curve) suggest that this dose of deoxyglucose severely inhibited disposition of the glucose load.

Table I reveals blood glucose response of fasted intact rats injected with 400 mg/kg of 2-deoxyglucose after intial glucose determination. A significant rise in glucose concentration compared to controls occurred between 60 and 120 minutes. Adrenal demedullated rats showed no hyperglycemic response to this dose of deoxyglucose, suggesting that hyperglycemia which occurs in intact rats resulted from epinephrine outflow from the adrenal medulla. Intact rats injected intravenously with dihydroergotamine 15 minutes before deoxyglucose also exhibited no response, lending confirmation to the epinephrine hypothesis.

The glucose tolerance curve of adrenal demedullated rats injected first with 400 mg/kg of 2-deoxyglucose followed by a glucose load is shown in Fig. 1. In contrast to the response of intact rats, there was no significant difference between demedullated rats and those given saline followed by a glucose load.

Discussion and Summary. Despite unquestionable evidence from the work of others that 2-deoxyglucose inhibits glucose oxidation in isolated tissues and eviscerated rabbits there was no impairment of disposition of a glucose

TABLE I. Blood Glucose Response in Groups of Fasted Rats to Intravenous 2-deoxyglucose.

Group	No. rats	Minutes 0	15	60	120	180
Deoxyglucose, 400 mg/kg	18	90 ± 2*	97 ± 2	99 ± 3	112 ± 5	143 ± 9
Saline controls	13	88 ± 2	79 ± 4	82 ± 2	83 ± 2	82 ± 3
Demedullated rats						
Deoxyglucose, 400 mg/kg	7	84 ± 2		87 ± 2	83 ± 4	82 ± 4
Intact rats						
DHE, 1 mg/kg I.V. after 1st blood	4	74 ± 2	76 ± 2	76 ± 4	76 ± 1	76 ± 3

* Stand. error.

load in adrenal-demedullated anesthetized rats pre-treated with large doses of deoxyglucose. This suggests that other pathways of glucose disposal must be utilized which compensate for inhibition of oxidation. These avenues of glucose disposal will be explored. Inhibition by DHE of the hyperglycemia which results from injection of deoxyglucose into intact fasted rats suggests that this glucose analogue is capable of stimulating release of epinephrine from the adrenal medulla. The lack of hyperglycemia following deoxyglucose injection in adrenal demedullated rats lends support to this hypothesis.

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Mechanism of Inactivation of Serum Oxidase (Ceruloplasmin) Activity by Ultraviolet Radiation. (24729)

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This paper is concerned with the effect of ultraviolet radiation on oxidase activity of ceruloplasmin, the copper containing protein in blood. Ceruloplasmin has a molecular weight of approximately 151,000 and 8 atoms of copper/molecule(2,3). It contains over 90% of copper of blood and is thought to be the copper storage and transport protein. That it also has oxidase activity is interesting. The exact enzymatic role of this copper protein is unknown at present although there have been reports that it can oxidize serotonin(4,5). Data are presented to show that as oxidase activity of serum or ceruloplasmin is destroyed by UV radiation, bound copper in the enzyme is changed resulting in increased amount of "unbound" copper.

Materials and methods. Total copper(6) and direct copper(7) determinations were made by the method of Gubler *et al.* A stainless steel water bath was constructed to hold Petri dish 5 cm in diameter. Ice water at 0°C and not varying more than 0.1° was continuously circulated through water bath. The solution to be irradiated with ultraviolet light was placed in the Petri dish. After temperature equilibration was established, the sample was exposed to UV radiation for definite time interval. A Mineralight model V-41 ultraviolet lamp (Ultraviolet Products, San Gabriel, Calif.) was used as ultraviolet (UV) source. The UV source was mounted above the solution so that it could be moved in vertical or horizontal direction. The lamp was placed 2.5 cm above the solution. At this height the intensity of radiation striking the solution was $[1.63 \pm 0.09] \times 10^4$ ergs/minute/mm^2 as determined by uranyloxalate actinometric method(8). Value of quantum yield used in the calculations was 0.60(9). Serum volumes were kept at 6 ml permitting thin uniform layers to be irradiated (3 mm). With purified ceruloplasmin, volumes of 1 ml and 3 ml were used. Evaporation was not noted either with ceruloplasmin or serum. After various samples were irradiated, they were permitted to warm up to 25° C. Aliquots were then analyzed for oxidase activity, total copper, and direct reading copper. Copper free glassware and reagents were used throughout. Serum samples were obtained from staff personnel. Limited amounts of ceruloplasmin were kindly donated by Upjohn Co., Ortho Research Fdn., and Red Cross Blood Program. *Assay.* When N,N-dimethyl-p-phenylenediamine dihydrochloride (DPP) reacts with serum or ceruloplasmin, the substrate is oxidized to a red colored com-