the serum pH of this patient to 3.4 resulted in recovery of only Type I amylase after Biogel P300 filtration. When the pH of the amylase-free fractions isolated by such filtration was restored to 7.2, the fractions incubated with normal human serum, and the mixture then filtered on Biogel P300, Type II as well as Type I amylase were recovered.

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Protection of Neonates against Whole-Body Radiation by the Administration of a Single Emulsified Injection of a Lipopolysaccharide During Pregnancy (33828)

SAMUEL J. PRIGAL
(Introduced by R. Rugh)
Department of Medicine, New York Medical College, Flower-Fifth Avenue Hospital, New York

In a previous publication it was reported that a purified endotoxin, a lipopolysaccharide (LPS) derived from *E. coli* could be given safely to mice if injected in a water-in-oil emulsion. Two to four times the LD_{50} dose was given with minimum lethality owing to the slow and prolonged release of the LPS from the mineral oil emulsion. The mice treated in this manner showed marked and prolonged resistance to challenges with a sarcoma—180 implant and a lethal infection with a staphylococcus (1). Experiments using whole-body radiation (WBR) as a challenge was, therefore, begun following the reports of Smith and her collaborators (2) and Zweifach and his collaborators (3), in which endotoxin was shown to be radioprotective. Additional observations by Smith *et al.*(4) and Ainsworth (5) and Hanks and Ainsworth (6) have confirmed and extended these observations.

In an experiment recently reported we obtained evidence suggestive of protection against lethal WBR in mice for approximately 1 month after a single injection of the LPS (7).

In other unreported experiments designed to see if the fetus could be protected against WBR the emulsified LPS was injected into CF_1 pregnant mice to determine their tolerance and it was found that 100 μg could be given without inducing abortion or killing the pregnant mouse which seems to be more vulnerable to the LPS. During the course of these studies a group of 11 neonates from a single litter became available and these were

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challenged with radiation (CF_1 neonates as shown in Table II). Survival, when radiated with 400 R, was so striking that a larger study with neonates was undertaken, and is the subject of this report.

Materials and Methods. Animals. Two strains of pregnant mice were used, CF_1 (Carworth) and Swiss-Webster (Manor). The dams were housed in individual cages and at birth continued to nurse their young in the same cage. They were fed Purina laboratory chow pellets and provided with water *ad libitum*. They were kept in air-conditioned quarters at 20–25°.

Lipopolysaccharide. The LPS was obtained from Difco and was prepared by the Boivin method from *E. coli* 026:B6.

Emulsification. The oil selected for the external phase was Drakeol 6VR, a purified mineral oil (Pennsylvania Refining Co., Butler, Pa.) and the basic emulsifier was Arlacel A, a purified mannide monooleate (Atlas Powder Co., Wilmington, Del.). More rapid emulsification and more stable emulsions were produced by the addition of glyceryl monooleate (S1097) obtained from Glyco Chemicals, New York, N. Y. The following proportions of ingredients for the oil phase were employed (ml): oil, 88, glyceryl monooleate, 2, and mannide monooleate 10.

Emulsor. An electric shaker, described in detail elsewhere, (8) which shakes at about 3000 rpm and moves in three distinct planes, was used for emulsification. (The Wig-L-Bug shaker no. 6000 is made by the Crescent Dental Mfg. Co., 1889 So. Pulaski Road, Chicago, Ill.)

Radiation. The radiation was provided through the kindness of Dr. Roberts Rugh, at the Radiobiology Laboratories of the College of Physicians and Surgeons, Columbia University, New York. Radiation emanates from two tubes, one above and one below the table, on which the animals are placed in a plastic cage. This assures rapid and equal radiation. The machine has a kVp of 184, mA 30 with filtration of Cu of 0.25 mm and Al of 0.5 mm.

Procedure. The emulsified LPS was given subcutaneously in doses ranging from 50 to 100 μg on or about the day 15 of gestation. In the case of the CF_1 mice, pregnancy was timed to a mating period of 2 hr under laboratory control. The Swiss-Webster strain of pregnant mice were obtained commercially so that the exact time of impregnation is not assured, although presumably they were all impregnated on the same day.

Seventeen dams of Swiss-Webster strain were divided into 5 groups. Three groups of 4 dams received respectively 50, 75, and 100 μg of emulsified LPS subcutaneously on or about day 16 of pregnancy. The remaining 5 served as controls; they were not injected with LPS. Some of their progeny (P-16 and 17) were radiated while others (P-15) were not. There were no immediate abortions or death nor for the ensuing crucial period of 72 hr after injection.

The estimated gestation period varied from 17 to 21 days. The number of neonates harvested from each dam varied from 0 to 13. The lowest yield came from the dams injected with 100 μg of LPS. There were fewer live births, and these died rapidly after birth so that only 5 of 21 neonates remained for radiation exposure. One dam (no. P11) resorbed her pregnancy completely. The 3 dams (P13, 14 and 15) serving as the nontreated, nonradiated controls yielded 33 neonates but only 11 of these survived the first 5 days. This would suggest that factors other than the LPS may have also operated in keeping the untreated neonate survival at a low level. The numbers of animals were too small to draw any definite conclusions, however.

Altogether 61 neonates from dams injected with LPS were ultimately available for radiation. Since births were irregular and all radiation was given on the same day, the day of radiation for each neonate varied from 3 to 7 days after birth. Likewise exposure to the LPS by the neonate during gestation was estimated to vary from 1 to 5 days. Table I summarizes this data.

Each pregnant mouse was housed separately following injection through birth. On or about the day 5 the neonates in groups of 5 were challenged with radiation, usually with

TABLE I. Effects of Emulsified LPS Injected during Pregnancy of Mice.

LPS dose (μg)	Mouse	Day of delivery	No. live	No. of still-births	Age at radiation (days)	No. of days exposed to LPS	No. of neonates for radiation
50	P-1	19–20	10	1	4–5	3–4	8
	2	18	13	3	NS[a]	2	0
	3	18	11	0	5	2	10
	4	17	9	1	6	1	8
	Totals		43	5			26
75	5	17–18	12	0	5–6	1–2	12
	6	21	9	0	3	5	8
	7	19	9	0	NS	—	0
	8	21	13	0	3	5	10
	Totals		43	0			30
100	9	17	8	8	NS	—	—
	10	18	8	0	NS	—	—
	11	—	—	—	—	—	—
	12	19	5	0	6	3	5
	Totals		21	8			5
0	13	19	9	0	NS		0
	14	19	13	2	NS		0
	15	19	11	0	Not radiated		11
	Totals		33	2			11
0	16	21	10	0	5		10
	17	21	9	0	5		9
	Totals		19	2			19

[a] NS = no survivors.

400 R except for one experiment in which 1000 R was given. Daily death checks were made for 30 days.

Results. The neonates born of dams treated with the LPS showed a high survival rate when given 400 R, whereas all neonates from nontreated dams were uniformly dead by the day 8 postradiation. There were no survivors of treated neonates exposed to 1000 R. Although the CF_1 neonates showed 100% survival, the number of animals treated was too small to draw firm conclusions. In the Swiss-Webster strain the survival rate was less dramatic, but nevertheless, substantial. Survival rates of 50 and 40% were obtained, respectively, when 75 and 50 μg of the LPS was given. Surprisingly, in this strain of mouse, 100 μg of LPS highly effective with CF_1 mice, was not protective. Perhaps this is expressive of a strain difference of tolerance to the endotoxin. It was noted in the Swiss mice that they did not tolerate the 100 μg of LPS well, as evidenced by reduced births and stillbirths so that only 5 neonates were available for radiation from 4 dams. This contrasted sharply from the substantial numbers of neonates (26 and 30) obtained from dams treated with 75 and 50 μg of the LPS.

Of 66 neonates exposed to radiation of 400 R, 31 were alive on day 30, an overall protection of 46.9%. This is significant ($p < .01$ by the chi-square test). The results are summarized in Table II.

Discussion. Radiation with 400 R was uniformly lethal for all unprotected neonates. Therefore any survival of treated neonates similarly exposed is significant. Survival rates of 40–100% have been observed in these experiments. Such variations need explanation. Perhaps this is expressive of strain differences. The CF_1 strain, it would appear, tolerates the LPS better. But one should not

TABLE II. Survival of Neonate Mice Exposed to Lethal Whole-Body Radiation.

Mouse strain	LPS (μg)	No. of neonates	Radiation dose (R)	Age at radiation (days)	30-day survival (no.)	30-day survival (%)
CF-1[a]	100	6	400	5	6	100
	100	5	1000	5	0[b]	0
Control	0	21	400	5	0[b]	0
Swiss-Webster[c]	50	26	400	5–7	13	50
	75	30	400	3–7	12	40
	100	5	400	5	0	0
Controls	0	11[d]	0	—	10	90.9
	0	**19**[e]	400	**5**	0[f]	0

[a] CF-1 Mice were mated for 2 hr and time of impregnation was determined; injection of LPS was given on day 15 of pregnancy.

[b] Mortality 100% by day 8.

[c] Swiss-Webster pregnant mice available from commercial source; time of pregnancy not accurately ascertained; injection of LPS given at estimated day 16 of pregnancy.

[d] Represents neonates of dams P-13, 14, and 15 in Table I.

[e] Neonates from dams P-16 and 17 in Table I.

[f] Mortality 100% by day 5.

overlook the unequal exposure of the embryos to LPS during gestation in the various groups, in view of the varying days of delivery. Furthermore, although most of the neonates were 5 days old when radiated, some were only 3 days old, perhaps making them more vulnerable to radiation; a few may have been more resistant being 1–2 days over 5 days old. All these factors could have modified the survival rates. To evlauate these variables a larger series of mice with accurately timed pregnancies would have to be employed. Despite these variables sufficient survival was encountered in the protected neonates to indicate that LPS given on or about day 15 of gestation exerts some degree of protection against whole-body radiation (WB R) for the neonate on or about the fifth postnatal day.

The mechanism whereby LPS affords protection against WBR has not been elucidated. In contrast to the chemical protectors such as the sulfhydryl compounds which must be present at the time of radiation, the effect of LPS is biologic, and does not require the immediate presence of LPS. It has a striking effect on the reticuloendothelial system. In unreported studies, emulsified LPS given in a 1-mg dose to mice produced enlarged livers and spleens, which were weighed 1 month after injection. In the case of the spleen, the increase in weight may be enormous, approaching 300% as compared with the spleen weights of normal mice. Perhaps it is the total increase in cells of the reticuloendothelial system (RES) particularly the blood-forming elements which exerts the protective effect. This "cellular" explanation for the protective phenomena has at various times been challenged by the concept of a mediating humoral mechanism. In this connection the well known work of Jacobson and his associates (9) must be mentioned along with the less known but more recent studies of Reichard (10), Berenblum *et al.* (11) and Hanna *et al.* (12).

The reported observations in this study in which the LPS with an approximate molecular weight of one million was given during gestation and yet was protective for the neonate would support the concept of a humoral protective factor. This view cannot be completely accepted as yet, since there is the possibility that despite its large molecular size, the LPS may have passed the placental barrier. Further studies elucidating the mechanism are planned.

The exposure of the unprotected neonate

to 400 R was 100% lethal. In view of this, the survival from such a severe challenge by the treated neonates must be considered as striking and significant. It may indicate that these neonates may possibly also resist infection (viral and bacterial) and the induction of cancer by an implant or through a carcinogenic virus.

Conclusion. A lipopolysaccharide (LPS) derived from *E. coli* was administered to mice during gestation yielding neonates that were highly resistant to lethal whole-body radiation. The LPS was given as an emulsion in mineral oil: the slow release from the emulsion eliminated the abortive and toxic effect of the LPS and prolonged its effective action.

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