monocytes to thrive and selectively survive in these cultures.

Summary. The response of peripheral WBC cultures to various endotoxins was compared to unstimulated control cultures. Three normal subjects with low antibody titers to *S. typhosa, S. enteritides* and *E. coli* were used as cell donors. These endotoxins produced lymphocyte transformation, DNA synthesis as measured by TdR-H^3 incorporation, occasional mitoses, and enhanced monocyte survival in their *in vitro* WBC cultures. In contrast, endotoxins derived from *pseudomonas* and *S. abortus equi* which are relatively nonantigenic were toxic when added to leukocyte cultures and resulted in little or no lymphocyte proliferation.

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Isogeneic Marrow Infusion Following Nitrogen Mustard in the Dog.* (30034)

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In several species it has been demonstrated that death from marrow failure following lethal irradiation can be prevented by an infusion of isogeneic marrow cells(1). Studies on the dog have shown that one billion or more nucleated autologous marrow cells following 1200 r whole-body irradiation results in sur-

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TABLE I. Survival Data of Dogs Given Large Doses of Nitrogen Mustard With or Without an Infusion of Autologous Marrow.

Group	Nitrogen mustard schedule	Dog No.	Autologous marrow	Survival or days to death
A	1.5 mg/kg, single dose	738	No	4
		820	"	Survived
		898	"	Survived
		844	Yes	4
		897	"	6
		900	"	Survived
		878	"	6
B	0.5 mg/kg, 3 doses given at 4 hr intervals, total dose 1.5 mg/kg	734	No	4
		837	"	4
		899	"	Survived
		826	"	7
		831	Yes	4
		857	"	4
		835	"	Survived
		839	"	5
		852	"	4
		840	"	4
C	0.3 mg/kg, 5 doses given at 24 hr intervals, total dose 1.5 mg/kg	918	No	Survived
		919	Yes	Survived
D	0.5 mg/kg, 5 doses given at 24 hr intervals, total dose 2.5 mg/kg	928	No	1
		926	"	5
		921	"	5
		908	"	3
		927	Yes	2
		923	"	3
		922	"	5
		924	"	5

vival of at least 90% of the animals(2,3).

The value of isogeneic marrow infusions as a means of avoiding the lethal effects of marrow failure following chemical agents is uncertain. Such infusions may be helpful after myleran(4) and Thio TEPA(5). Conflicting reports have appeared concerning the value of isogeneic marrow infusion following toxic doses of nitrogen mustard(6-10). This report describes some experiences with dogs given large doses of nitrogen mustard with or without a subsequent infusion of isogeneic marrow.

Methods. The dogs employed in these studies were young beagles or mongrels, 6 to 18 months old, free of worms and vaccinated against distemper and hepatitis. Supportive care including antibiotics, fluids and isolation was as described elsewhere for irradiated dogs(11).

Nitrogen mustard (HN2, mechlorethamine, Merck, Sharp and Dohme) was dissolved immediately before use and administered intravenously.

Autologous marrow was procured from surgical windows in the femurs and processed as previously described(2,11). Marrow was obtained shortly before administration of HN2, kept at 4°C and infused intravenously one hour after the last dose of HN2 (Groups A and B). The marrow of the dogs in Groups C and D was preserved in dimethyl sulfoxide at −80°C(11). One to 4 × 10^9 nucleated marrow cells were employed in each instance, a dose previously demonstrated to be adequate for irradiation protection(2).

Results. Dogs given 0.6 or 0.8 mg HN2 per kg survived. Of 12 dogs given 1.0 mg HN2 per kg, 9 survived. These animals showed a severe leukopenia by the fourth day with subsequent rapid return to normal white blood cell counts by 8-10 days. Three animals died on the 4th to 5th day with bloody diarrhea. Autopsy demonstrated coccidiosis involving the intestinal mucosa. In view of these results larger doses of HN2 were employed in the following marrow protection experiments.

Group A (Table I): Seven dogs received a single dose of 1.5 mg HN2 per kg. Three dogs were not given marrow infusions, 2 survived. Four dogs were given marrow infu-

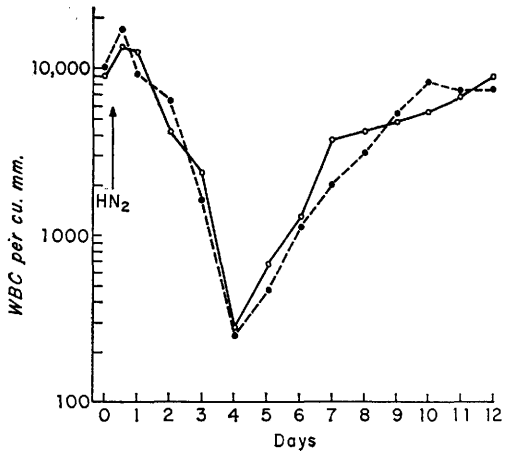

FIG. 1. Average leukocyte counts of dogs given 1.5 mg HN2 per kg. ○——○ 3 animals not given autologous marrow (one died on 4th day). ●---● 4 animals given autologous marrow (one died on 4th day; 2 died on 6th day).

sions, 1 survived. Fig. 1 compares the average white blood cell count of the dogs given marrow and the dogs given no marrow. The survivors demonstrated a rapid recovery in white blood cell count whether or not they had received a marrow infusion.

Group B (Table I): Ten dogs received 1.5 mg HN2 per kg divided into 3 doses of 0.5 mg per kg given 4 hours apart. Of 6 dogs that received an autologous marrow infusion, 1 survived. Of 4 dogs that did not receive marrow, 1 survived. Leukocyte counts of the 2 survivors showed a nadir of 190 and 230 cells per cu mm on the 4th day with a rapid return to normal as in the dogs of Fig. 1.

Group C (Table I): Two dogs were given 0.3 mg HN2 per kg daily for 5 days. One received an infusion of stored autologous marrow. Both dogs survived. The nadir of the leukocyte count occurred on the 6th day after the last dose of HN2 with prompt recovery of both dogs thereafter.

Group D (Table I): Eight dogs were given 0.5 mg HN2 per kg daily for 5 days. One hour after the last dose of HN2, 4 dogs were given an infusion of their own stored marrow. All 8 dogs died 1 to 5 days after the last dose of HN2. White blood cell counts showed no difference between the 2 groups.

Discussion. The results presented here indicate no protective effect of isogeneic marrow following toxic doses of nitrogen mustard in the dog. Although other schedules of drug administration might disclose some protection by isogeneic marrow, it seems unlikely that any significant increment in the permissible dose of the drug will be achieved by this means.

A marrow infusion may be expected to exert a protective effect against drug toxicity only where the toxic effect is primarily on the marrow. In our laboratory irradiated dogs that die of marrow failure generally die 8-10 days after exposure. Inspection of Table I shows that dogs that died of HN2 toxicity generally died after a shorter time interval, indicating toxic effects on other organ systems in addition to marrow. All these animals had diarrhea of varying severity, but dehydration was prevented by parenteral fluids and electrolytes. Complete autopsies did not disclose a specific cause of death.

The rapid recovery of marrow function after toxic doses of HN2 (Fig. 1) is of particular interest. A rapid recovery of this type is seen in irradiated dogs that have successful marrow grafts, a recovery presumed to be due to stem cells in the infused marrow(1,3). The recovery demonstrated in Fig. 1 suggests, therefore, that relatively undamaged stem cells are present in the marrow following HN2. These cells are able to bring about a rapid marrow recovery provided the animal lives long enough for the recovery to occur. If stem cells are relatively undamaged by HN2, it would appear reasonable to expect no protective effect from isogeneic marrow infusions following HN2.

Summary. Dogs given nitrogen mustard in near-lethal and supra-lethal amounts were not protected against the toxic effects of the drug by an infusion of isogeneic marrow. The rapid marrow recovery exhibited by the survivors not given marrow indicated that stem cells are relatively undamaged by toxic doses of nitrogen mustard.

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Effect of Sodium Taurocholate on Fat Malabsorption Induced by Feeding Unheated Soybean Proteins.* (30035)

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Chicks fed diets containing unheated hexane-extracted soybeans absorb fat poorly(1, 2). The portion of the soybean responsible for causing the poor absorption of fat is present in a group of proteins, soluble at pH 4.4 and readily extractable from the soybean, sometimes termed "whey" proteins(3). The causative agent in the soybean is heat labile and only affects chicks under 2 to 3 weeks of age. Chicks 4 weeks old or older normally show little impairment of fat absorption when fed unheated soybean meal preparations(2).

The experiments reported here show that the effect of these soybean proteins on fat absorption in young chicks can be counteracted by including the bile salt, sodium taurocholate, or lecithin in the diet fed to chicks.

Experimental. Male Rhode Island Red X Barred Plymouth Rock crossbred chicks from a commercial hatchery were used in all experiments. The chicks were housed in thermostatically controlled battery brooders with raised wire floors and feed and water were supplied *ad libitum*. The absorption of dietary fat was determined by the procedures described by Renner and Hill(4). Excreta collections were made on the last 4 consecutive days of the 2-week experimental period. The diet used was the same described by Nesheim *et al*(2) and contained soybean meal, glucose, soybean oil, DL-methionine, glycine, vitamin and mineral supplements. Unheated soybean meal was included in the experimental diet at the expense of an equal weight of heated soybean meal. The unheated soybean meal consisted of dehulled, hexane-extracted flaked soybeans which had been subjected to a minimum of heat treatment. The heated soybean meal was commercially prepared 50% protein soybean meal. Brambila *et al*(1) conclusively showed that the factor in soybeans responsible for inducing poor fat absorption in chicks was heat labile.

Total bile acids in bile were determined according to the method of Levin and coworkers(5).

Results. The first experiment was conducted to determine whether addition of sodium taurocholate or lecithin to a diet containing unheated soybean meal would affect the absorption of fat by chicks fed this diet. The results are shown in Table I.

Chicks fed the diet containing heated soybean meal absorbed 94% of the ingested fat. Those fed the diet containing unheated soybean meal absorbed only 38% of the ingested fat. Chicks receiving the diet containing unheated soybean meal and 0.6% sodium taurocholate absorbed fat to the same extent as the control chicks receiving the diet containing heated soybean meal. The chicks receiving the diet containing unheated soy-

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