infusions of 1.25 mg/kg will produce thrombosis within 2 minutes. Chicken serum is inactive in the rat.

Whether the difference between the 2 human serum lots was due to real differences between the lots of serum or to variation between the two groups of assay birds is not known. Until the sources of variation are known, this sort of assay should be done on a single group of birds within a short period of time.

Gross alterations in the plasma lipids of assay birds failed to cause any differences in the clotting rates. Until infused sera from patients with hyperlipidemias or recent thrombotic episodes can be assayed, one cannot exclude a role for lipids in this system. The system described is suitable for quantitation and use of this approach may facilitate the evaluation of the role of lipids in intravascular thrombosis.

Summary. (1) A method of producing serum-induced intravascular thrombi has been adapted to use in a convenient laboratory animal, the chicken. (2) Dose-response curves can be constructed and statistically compared. (3) The factors influencing the clotting rates are not all known, but alterations in serum lipids in the assay animals did not influence the results.

1. Duguid, J. B., *Lancet*, 1954, v1, 891.
2. Hartroft, W. S., Thomas, W. A., *J. Am. Med. Assn.*, 1957, v164, 1899.
3. Poole, J. C. F., *Brit. Med. Bull.*, 1958, v14, 253.
4. Hashim, S. A., Clancy, R. E., *N. Eng. J. Med.*, 1958, v259, 1115.
5. Wessler, S., *J. Clin. Invest.*, 1955, v34, 647.
6. Wessler, S., Reimer, S. M., *ibid.*, 1960, v39, 262.
7. Wright, I. S., *Ann. Int. Med.*, 1959, v51, 841.
8. Carpenter, K. J., Gotsis, A., Hegsted, D. M., *Clin. Chem.*, 1957, v3, 233.
9. Hegsted, D. M., Gotsis, A., Stare, F. J., *J. Nutrition*, 1960, v70, 119.
10. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
11. Pizzi, M., *Human Biology*, 1950, v22, 151.
12. Didisheim, P., Hattori, K., Lewis, J. H., *J. Lab. Clin. Med.*, 1959, v53, 866.

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Survival of Calves Treated with Autologous Bone Marrow After Exposure To Lethal Dose of Whole-Body Irradiation.* (26096)

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In conjunction with earlier experiments on induction of aplastic anemia in calves by trichloroethylene-extracted soybean oil meal (1) or by S-(dichlorovinyl)-L-cysteine(2), we attempted, without success(3), to prevent development of the blood dyscrasia by administration of D,L-batyl alcohol. Inasmuch as the toxic agent, if given in sufficient amounts and without therapeutic treatment, needs to be administered for only a short period of time(2,3) to damage the blood forming organs irreversibly, it appeared possible that hematopoiesis might be restored by intravenous injection of homologous bone marrow or other hematopoietic tissues. Bone marrow transplants have been effective in several species for recovery of hematopoietic function after the bone marrow had been

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damaged by lethal doses of X-, gamma- or neutron-irradiation(4). In mice homologous fetal liver has been reported(5) to afford greater protection against radiation damage than adult homologous bone marrow. Administration of bone marrow has also been used against the toxic effects of some "radiomimetic" compounds which damage the bone marrow. Thus, treatment with isologous bone marrow afforded protection of rats against 1,4-dimethanesulfonoxybutane(6,7) and of mice against one of its higher homologs(8). Tran Ba Loc *et al.*(9) found that isologous but not homologous bone marrow or spleen protected mice against the nitrogen mustard, methyl-bis-(2-chloroethyl) amine. Kawamoto(10) reported that treatment of mice with isologous bone marrow gave some protection against toxic effects of urethan.

In our experience(11) with calves in which severe bone marrow damage was induced by feeding of ½ lb trichloroethylene-extracted soybean oil meal per day per 100 lb bodyweight for 10 days(3), the intravenous injection, on the second or fifth day after withdrawal of the toxic feed, of homologous bone marrow in amounts containing 1.1 to 4.9 x 10^{10} nucleated cells failed to alter the course of subsequent fatal blood dyscrasia.

Inasmuch as there is no previous record, to our knowledge, of attempts to transplant bone marrow in bovine animals it was important to determine whether this procedure could afford in this species, as it has in several others, protection against radiation-induced aplastic anemia.

Procedures. Female Holstein calves weighing initially about 50 kg were managed, conditioned and then exposed to a lethal dose of 250 roentgens (tissue exposure dose at the midline) gamma-radiation under exactly the same conditions as those previously described (12). Hematologic examinations were made daily for 3 months, later less often, with blood collected from a jugular vein. Tissues used for therapeutic treatment were collected and injected as follows:

Fetal tissues. The fetuses, with an estimated age of 3½ to 4½ months were obtained at slaughter in a local abbatoir and brought to the laboratory for removal of tissues under aseptic conditions within one hour after death of the cow. Liver, spleen and thymus, with average weights of 31.9, 2.1 and 1.3 g respectively were homogenized gently, for 10 seconds, with a small volume of cold Geys'-citrate solution(13), strained through a nylon gauze and the fluid was injected, intravenously, about 19 hours after completion of the irradiation. The injected suspensions contained from 7.8 x 10^9 to 9.1 x 10^{10} nucleated cells with an average of 2.9 x 10^{10}, about half of which were hemic cells.

Homologous bone marrow. Immediately after slaughter of normal female Holstein calves, bone marrow was squeezed with the aid of a mechanics' vise from the ribs and from segments of the sternebrae and vertebral column. The marrow was collected in cold Geys'-citrate solution and filtered through a sterile nylon gauze to remove fat and gelatinous clots. The filtered suspension was injected intravenously into calves about 19 hours after completing their irradiation. The injected bone marrow contained from 2.1 to 4.7 x 10^{10} nucleated cells.

Autologous bone marrow. This was collected with heparinized syringes by biopsy of 18 to 20 sites on the sternum and ribs, about 2 hours before the animal was irradiated. The collected marrow was diluted with cold Geys'-citrate solution and stored cold until injected, intravenously, within 1 hour after completion of the irradiation.

All injected suspensions contained 500 units of penicillin per ml. They were injected at a rate of about 5 ml per minute. The volumes of the injected suspensions ranged from 50 to 375 ml.

Results. In general the calves tolerated the injections well; only 2 cases of temporary respiratory distress were observed after treatment with homologous bone marrow. As shown in Table I all calves which received homologous bone marrow, fetal tissues or no therapeutic treatment died about 20 days after irradiation, or were slaughtered in moribund condition. There was no evidence that homologous bone marrow or the fetal tissues modified in any way the course of the "bone marrow syndrome" or radiation damage. No indications were obtained that the injected

TABLE I. Effect of Bone Marrow or Fetal Tissues on Survival of Calves Treated with 250 r Whole-Body Gamma Radiation.*

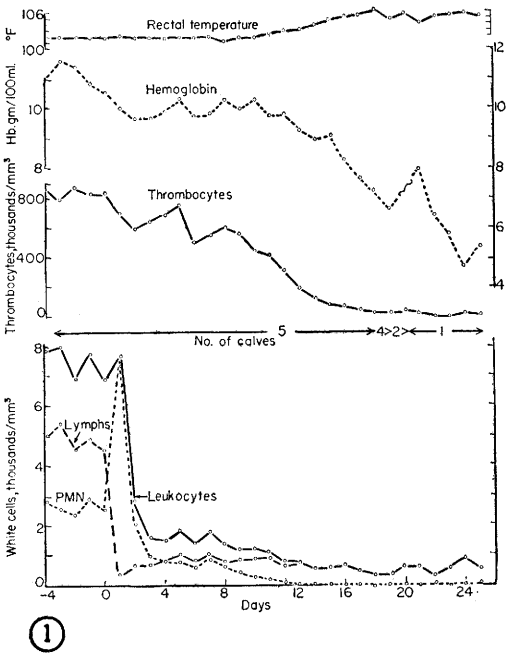
No. of calves	Therapeutic treatment, I.V. Substance	Mean No. nucleated cells	Mean days until death
10‡	None	None	19.4 ± 2.9†
5	Homologous bone marrow	3.8×10^{10}	18.4 ± 1.6
5	Mixture of homologous fetal liver, spleen, thymus	2.9×10^{10}	20.2 ± 3.1
6	Autologous bone marrow	1.2×10^{9}	23.1 ± 4.8
9	*Idem*	1.5×10^{9}	Survived

* Calculated as tissue exposure dose at the midline.
† Stand. dev.
‡ Animals referred to previously(12).

cells were able to proliferate in the irradiated host and repopulate the hematopoietic centers. Period of survival after irradiation was not prolonged by the treatment.

In five of the 15 calves which received autologous bone marrow the blood dyscrasia also took a fatal course. The changes in the hematologic values of these 5 calves are shown in Fig. 1. They coincide essentially with those previously reported(12) for the uninjected calves, and with those observed in the calves treated with homologous bone marrow or fetal tissues.

In contrast to this, 10 calves which were also injected with autologous bone marrow, after a severe hematologic crisis between the 14th and 20th day made a hematologic recovery as shown in Fig. 2. Their rectal temperatures never reached the high values of up to 107° observed in calves which failed to recover. One of these animals started to show rapid hematologic recovery involving all blood elements after the 22nd day but died from rupture of a coronary vessel, with fatal hemorrhage on the 28th day; it is not included in Table I in the group shown to have survived the bone marrow syndrome. In the other animals there was a gradual increase in lymphocytes and the polymorphonuclear leukocytes as shown in Fig. 2. After the 40th day the thrombocyte counts became stabilized at about 450,000 per cmm which is somewhat subnormal for calves of this age. No external evidence of radiation damage



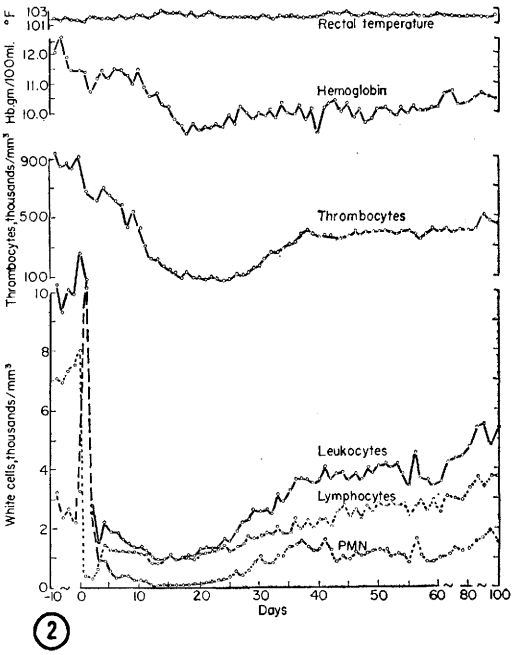


FIG. 1. Hematologic changes in "non-survivors" following exposure to lethal dose of gamma-radiation and I.V. inj. of autologous bone marrow.
FIG. 2. Hematologic changes in "survivors" following exposure to lethal dose of gamma-radiation and I.V. inj. of autologous bone marrow.

such as clouding of the cornea, depilation or change in hair color was ever observed.

The subsequent development and status of the calves which survived after treatment with a lethal dose of gamma-radiation and autologous bone marrow is of interest. After 60 days they were fed a ration of alfalfa hay supplemented with a grain mixture and kept outdoors most of the time. Since spring of 1960 they have been on pasture. One animal ingested a foreign body and was slaughtered because of ensuing traumatic pericarditis and reticulitis, 11 months after irradiation. The 8 other animals developed normally and had estrous cycles with regular intervals. They have been bred by artificial insemination and seven are now (19 to 27 months after irradiation) pregnant. At various times their blood counts have reached levels which are considered normal for heifers of this age. There has been a tendency toward thrombocytopenia in 4 of the animals with counts of 200,000 to 300,000 per cmm being recorded several times. No evidence of leukemia has been obtained. With the exception of 1 heifer which is somewhat gaunt, the animals appear to be normal.

Summary. Intravenous injection of homologous bone marrow or of a mixture of fetal liver, spleen and thymus after exposure to a lethal dose of whole-body gamma-radiation failed to protect calves against death from bone marrow damage. Nine of 15 calves treated with autologous bone marrow recovered after a severe hematologic crisis; 8 of these developed normally, showed no apparent signs of late radiation damage, had regular estrous cycles and 7 are now pregnant.

1. Schultze, M. O., Seto, T. A., Mizuno, N. S., Bates, F. S., Perman, V., Sautter, J. H., *Internatl. Soc. Hematol. Proceed. VIth Congress.* Grune and Stratton, N. Y., 1958, p. 794.

2. Schultze, M. O., Klubes, P., Perman, V., Mizuno, N. S., Bates, F. W., Sautter, J. H., *Blood,* 1959, v14, 1015.

3. Schultze, M. O., Perman, V., Bates, F. W., Sautter, J. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 470.

4. Congdon, C. C., in *Progress in Hematology,* 1959, v2, 21, Grune and Stratton, New York.

5. Urso, I. S., Congdon, C. C., Owen, R. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 395.

6. Weston, J. K., Maxwell, R. E., Lee, M., Finzel, J., Fisken, R. A., *Federat. Proc.,* 1957, v16, 377.

7. Dunjic, A., Maisin, J., *Rev. franc. d' études clin. biol.,* 1960, v5, 268.

8. Talbot, T. R., Elson, L. A., *Nature,* 1958, v181, 684.

9. Tran Ba Loc, Mathé, G., Bernard, J., *Rev. franc. d' études clin. biol.,* 1958, v3, 461.

10. Kawamoto, S., in *Radiation Biology and Cancer.* Univ. Texas Press, 1959, p. 203.

11. Unpublished experiments by the authors.

12. Schultze, M. O., Perman, V., Mizuno, N. S., Bates, F. W., Sautter, J. H., Isbin, H. S., Loken, M. K., *Radiation Res.,* 1959, v11, 399.

13. Gey, G. O., Gey, M. K., *Am. J. Cancer,* 1935, v27, 45.

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Trachoma Viruses Isolated in United States. 3. Strain specific effects of tetracycline and penicillin.* (26097)

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Members of the psittacosis—LGV—trachoma group of viruses differ from "true" viruses in being susceptible to the action of many antimicrobial drugs both experimentally and clinically. Long before trachoma viruses were first successfully cultivated(1) millions of persons in trachomatous areas were being treated with topical tetracycline or sulfonamide applied to the eye. Under the auspices of the World Health Organization extensive trials of various time-dose regimens

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