dosage that would be found if 2 different strains were used. The possible role of dosage effect in increasing the protection afforded irradiated mice by injection of homologous cells is discussed by Uphoff(8).

Summary and conclusions. Survival of lethally x-irradiated (BALB/c X A)F_1 hybrid mice was greatly prolonged by injection of leukemoid blood from (BALB/c X C3H) F_1 hybrid mice. Quantitative estimates indicate that many, if not all cells in lymph nodes or marrow of irradiated protected mice were derived from leukemoid blood.

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Molecular Weight and Plasma Substituting Effectiveness of 3 Plasma Expanders. (24816)

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On the assumption that only compounds of high molecular weight were capable of substituting for blood plasma, dextrans of high molecular weight were initially employed in treatment of shock. These dextrans gave rise to a high incidence of side effects which were attributed to high molecular weight of the compound(1). Wilkinson and Storey(2) essayed a dextran considered to be "highly fractionated" (65% of molecules weighing between 130,000 and 250,000). They found in normal volunteers that it still produced a high incidence of urticaria and vasomotor instability. Since then the molecular weight of clinically used plasma expanders has been greatly diminished without significant change in their therapeutic effectiveness. Most of those now in use have an average molecular weight between 30,000 and 40,000(3). The study of a new compound of much lower molecular weight was considered interesting since it might produce fewer side effects than reported for the higher polymeric expanders. In the present investigation 3 plasma expanders were compared as to their capacity to restore normal blood pressure and plasma volume as well as to prevent death from an experimentally produced hemorrhage.

Materials and methods. The low polymeric plasma expander studied was a partially methoxylated polymer of galacturonic acid whose molecular structure resembles that of dextran (Fig. 1). Its average molecular weight was only 6,200. This substance was used in a stabilized and buffered 1% solution in saline (Graplasmoid, L. I. F. E.). The relatively higher polymers employed for comparison were: 6% dextran in saline (Intradex, Glaxo) with average molecular weight of 40,000, and

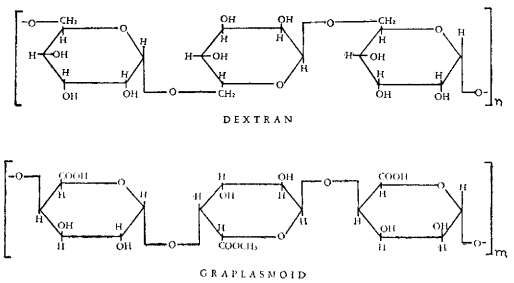

FIG. 1. Chemical structure of a moiety of dextran and Graplasmoid.

3.5% polyvinyl-pyrrolidone (PVP) in saline (Periston, Poulenc) with an average molecular weight of 28,000. As reference standard, heparinized whole blood obtained from test animal itself was used. For further comparison 2 crystalloid aqueous solutions, namely, 5% dextrose and 0.9% NaCl, were employed. All solutions were sterile and free of pyrogens. Since Rosenthal and Millican(4), in a review on the role of various fluids in traumatic shock and hemorrhage (287 references), conclude that there is no standard method for evaluating therapeutic value of plasma expanders and that tests in dogs are difficult to standardize because of lack of uniformity of race, weight, etc., in any adequately large population of animals, white male rabbits of a uniform strain weighing approximately 2 kg and guinea pigs weighing 0.5 kg were chosen for the present study. To determine the most appropriate amount of blood withdrawal ("hemorrhage"), preliminary experiments were conducted in which the animals were freely bled to death by opening one carotid artery and amount of blood shed was measured. Under these conditions average loss (± standard error), in 10 rabbits, was 30.6 ml/kg ± 0.98 ml/kg and 35.2 mg/kg ± 3.8 ml/kg, in 10 guinea pigs. In both species average bleeding time was 5 minutes. On the basis of these results 15 ml/kg (50% of the lethal blood loss in rabbits) was chosen as "standard hemorrhage." In series A, experiments in rabbits were performed after Hamilton *et al.*(5), by producing rapid standard hemorrhage (1 minute duration) from the carotid artery and replacing the blood lost by an equal amount of test fluid, injected immediately after hemorrhage (1 minute duration). The effectiveness of plasma expanders was evaluated by studying blood pressure over a carotid cannula for 2 hours, following experimental hemorrhage. All animals were heparinized, and anesthetized intraperitoneally with a combination of urethan (700 mg/kg) and pentobarbital (30 mg/kg). In series B, effectiveness of plasma expanders was evaluated by determining blood volume freely shed from opened carotid artery until death, 4 hours after standard hemorrhage and subsequent replacement of blood by the test fluid (*cf.* Lawson(6)). In additional groups, C and D, percent survival after partial replacement of blood by test fluid was studied. In series C, animals from series A were employed. After recording blood pressure for 2 hours, the carotid wound was sutured and the animal observed 24 hours. In series D, the standard hemorrhage and respective substitution of blood by test expander were made twice with an interval of 24 hours. Incidence of death was recorded for 24 hours after second hemorrhage. Studies on survival were also performed in guinea pigs (series E and F). After a severe hemorrhage of 30 ml/kg the volume of test expander injected was 10 ml/kg (1/3 of amount shed) in series E, while in series F it was equal to the amount shed (30 ml/kg). Survival was recorded for 24 hours after substitution of blood by the expander.

Results. (a) *Changes of blood pressure.* A rapid loss of 15 ml/kg of blood produced an abrupt fall of blood pressure 42 to 49% of normal. The immediate infusion of equal amount of any of the 6 test fluids rapidly restored blood pressure to near-normal levels (Fig. 2). Differences were not significant. This return, however, was followed during the next 10 minutes by drop in blood pressure, which varied greatly according to fluid used. When blood substitution consisted in autotransfusion, blood pressure, once restored after initial fall, did not essentially change. A

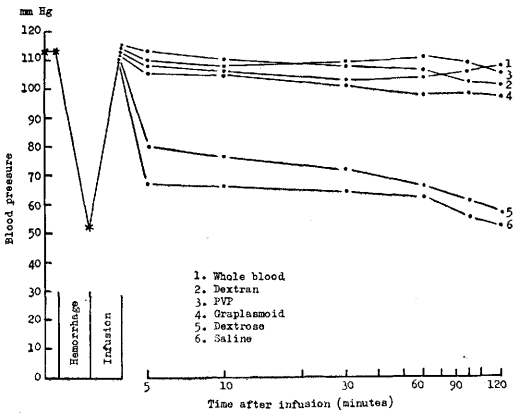

FIG. 2. Blood pressure changes after a rapid hemorrhage and an immediate infusion of a test fluid. Each point represents avg systolic pressure in groups of 8 to 15 rabbits.

slow decrease by about 10 mm Hg during the first hour was followed during second hour by complete return to original prehemorrhagic pressure. Behavior of blood pressure after dextran, PVP and Graplasmoid was not essentially different from that observed after a blood infusion. The final level after test period of 2 hours, was only slightly below that reached after blood. Even the largest difference, observed with Graplasmoid, the only one which was mathematically significant ($P = <0.05$) was not more than 15 mm Hg.

Quite different was the blood pressure curve after dextrose and saline. The rapid return to normal level after post-hemorrhagic drop was followed, within 5 minutes after infusion, by equally rapid drop by 35 and 45 mm, respectively. From this lower level the pressure continued to decline during 2-hour observation period, finally ending at the low post-hemorrhagic level. Differences from levels after blood infusion were significant ($P <0.01$).

TABLE I. Blood Volume Obtained from Rabbits Freely Bled unto Death, 4 Hours after Replacement of 15 ml/kg of Blood by a Plasma Expander.

Plasma expander	No. of animals	Avg ± stand. error (ml/kg)	Relative vol
Blood	5	28.9 ± 2.2	100
Dextran	6	27.7 ± 2.0	96
PVP	5	27.2 ± 2.1	94
Graplasmoid	6	26.5 ± 2.3	92
Dextrose	5	17.9 ± 2.2	62
Saline	5	17.2 ± 2.3	60
Untreated controls	5	17.1 ± 2.1	59

b) *Blood volume 4 hours after infusion.* Blood volumes shed in a lethal hemorrhage 4 hours after partial substitution of blood by a test fluid are presented in Table I. Results obtained after infusion of plasma expanders were different from those after infusion of crystalloid solutions. While total volume shed after administration of test plasma expander was not essentially different from that after autotransfusion (only 8% lower in the case on Graplasmoid), it was greatly diminished after administration of dextrose or saline.

c) *Post-hemorrhagic survival.* In a group of non-treated rabbits 62.5% of animals survived 24 hours after a single standard hemorrhage. Survival diminished to 25% when blood withdrawal was repeated 24 hours later. When the blood shed was replaced by dextrose or saline, practically the same results as in non-treated group were obtained (Table II). However when blood was replaced by one of the 3 colloidal expanders, more than 90% of animals survived both single and double hemorrhage.

TABLE II. Survival of Rabbits 24 Hours after a Single (Group A) and a Double (Group B) Experimental Hemorrhage Treated with Various Fluids. Amount of blood replaced by an equal volume of plasma expander was 15 ml/kg.

	Group A		Group B	
Plasma expander	Death	% survival	Death	% survival
Blood	0/8	100	0/8	100
Dextran	"	"	1/10	90
PVP	"	"	0/10	100
Graplasmoid	1/15	93	"	"
Dextrose	3/10	70	5/7	29
Saline	2/8	75	6/8	25
Untreated controls	3/8	62	"	"

The results in guinea pigs, after severe hemorrhage (30 ml/kg) are presented in Table III. When 10 ml/kg of a colloidal fluid were injected immediately after the hemorrhage, 60 to 70% of animals survived, and when amount of expander infused was the same as blood withdrawn, survival increased to 100%. Substitution of blood by dextrose or saline was less effective in protecting animals against death.

Discussion. According to our experiments a polymer of low molecular weight (Graplasmoid) had a plasma substituting capacity qualitatively similar to that of polymers of

TABLE III. Survival of Guinea Pigs 24 Hours after a Hemorrhage of 30 ml/kg Treated with Different Doses of a Plasma Expander.

	Infusion = 10 ml/kg		Infusion = 30 ml/kg	
Plasma expander	Death	% survival	Death	% survival
Blood	1/5	80	0/5	100
Dextran	3/10	70	0/10	100
PVP	2/5	60	1/5	80
Graplasmoid	3/10	70	0/10	100
Dextrose	3/5	40	3/5	40
Saline	4/5	20	4/10	40
Untreated controls	5/5	0		

higher molecular weight, such as dextran and PVP. Quantitative differences were rather small and are possibly devoid of biological significance. It seems that if a plasma expander has characteristics of a colloidal solution, large weight of molecules might not be an indispensable factor in restoring hemodynamic properties of blood plasma. Nevertheless, molecular size and shape must be also studied before a definitive conclusion on the influence of molecular weight upon effectiveness of a plasma expander can be drawn.

It now seems(7,8) that high molecular weight of compounds is not the only factor involved in production of side effects. Wilkinson(7) found that, in spite of the fact that dextrans of 42,000 or 38,000 average molecular weight produced a lesser incidence of side effects than still higher polymeric compounds (2), the 42,000-type produced even a lesser incidence than the 38,000-type, a difference the author attributed to the fact that 2 different strains of *Leuconostoc mesenteroides* were used to synthesize the 2 dextrans and may have produced molecules of somewhat different structure. Moeller(9) in another kind of assay also found that dextran of lower molecular weight produced less cutaneous reactions.

Dextran is a mixture of polymeric hexoses in which 1:6-alpha-glucoside links prevail. Kabat and Berg(8) suggested that the differences between precipitin values produced by different dextrans could be related mainly to the number of 1:6 links, rather than to molecular weight. For another antigenic phenomenon, *i.e.*, complement fixation, however, Hehre and Neill(10) found it greater with heavier molecules. Dextran and PVP, besides their antigenic properties, induce liberation of histamine(11), to which some of the side effects are ascribed. It has been demonstrated(12) that histamine release also increases with higher molecular weight and concentration of dextran. Dextrans of molecular weight below 14,000 as well as dextran sulphate of molecular weight below 10,000 did not induce histamine release(12). Furthermore, Walton(13) showed that toxicity of dextran sulphate could be decreased by diminishing its molecular weight. The "reactions" to plasma expanders —allergic reactions, agglutination, complement fixation, precipitation, histamine release, etc.—seem to be rather complex; they appear to depend upon various factors such as weight, size, shape, and structure of molecule and concentration of drug. Like dextran and PVP, Graplasmoid is used therapeutically as a plasma expander and has not yet been reported to produce any side effects described for higher polymeric plasma expanders. Because of its low molecular weight a low concentration of Graplasmoid (1%) is required to obtain an isotonic solution with plasma, whereas higher concentrations of PVP (3.5%) and dextran (6%) are required. This low concentration could be a contributing factor in diminishing side effects.

Summary. Capacity of 3 plasma expanders of different molecular weight to substitute blood plasma was studied. These included dextran (40,000), PVP (28,000) and Graplasmoid (6,200); they were compared with whole blood and with 2 non-colloidal solutions dextrose and saline. Changes in blood pressure and blood volume observed after partial replacement of blood by any of the 3 colloidal solutions were not essentially different from those observed after autotransfusion. Replacement by dextrose or saline solutions, however, resulted in significant changes. The non-colloidal solutions only slightly delayed onset of hemorrhagic shock. The plasma expander with lowest molecular weight was as effective as the 2 high molecular weight compounds. A greater number of animals survived partial or "total" replacement of blood with plasma expanders than survived replacement with dextrose or saline. In percent of survival, no significant differences were observed between the 3 plasma expanders.

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Pituitary Prolactin Levels in Laying, Incubating and Brooding Pheasants (*Phasianus colchicus*).* (24817)

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The ring-necked pheasant (*Phasianus colchicus*) in Wisconsin can be classified as an "indeterminate layer"(1). This classification is used for birds which lay until accumulation of eggs releases the incubation response. Pheasants in Wisconsin drop a considerable number of eggs prior to laying in clutch and incubating(2). The physiological control of initiation of incubation and broodiness has been studied. Riddle and Lahr(3) and Lehrman(4) indicated that progesterone induces incubation in ring doves. Only recently has progesterone been identified in birds(5,6,7). Association of prolactin with broodiness is well known(8,9,10). Crispens(11) was able to induce broodiness in hen pheasants prior to and during reproductive season by injecting prolactin. His criterion for broodiness was acceptance of chicks by a treated hen. One can postulate that initiation of incubation is controlled by prolactin. Actual relationships between pituitary prolactin levels during incubation and brooding is not well established. Understanding of various physiological changes during the reproductive period is necessary before activities of bird can be properly interpreted. We conducted this study to determine pituitary prolactin levels during various stages of incubation and brooding.

Materials and methods. Hen pheasants were autopsied in various stages of reproduction in 1955 and 1956. Anterior pituitary glands of some of these birds were removed, weighed and immediately frozen for prolactin bioassay. In 1955, 3 to 5 glands were chosen from birds in the following stages: laying; 8th, 16th and 20th days of incubation; and 3rd, 7th and 11th days after hatching. The same procedure was followed in 1956 when 3 to 5 glands were chosen from birds in both laying and non-laying condition 4th, 8th, 12th, 16th and 20th days of incubation; and 3rd, 7th and 11th days after hatching. In late July, immediately following breeding season, glands from birds in each stage of reproduction were pooled and homogenized in a glass homogenizer. The homogenate was diluted with distilled water to concentration of 20 mg fresh weight tissue/cc. These aqueous homogenates were injected intracutaneously over crop glands of 8-week-old white king pigeons, a modification of technic of Lyons and Page(12). With this method it is possible to test 2 homogenates in each bird, 1 for each crop gland. Prior to assay homogenates were coded. Crop glands to test each homogenate, were selected at random. Each preparation provided test material for at least 2 assay glands. Water placebo was injected over 4 crop glands to provide additional control. The spot over each crop gland around

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