cance of this finding is questionable in view of the small number of animals in this group. Castrate males had articular lesions as often and severe as intact males. They were significantly (p. $<.01$) larger (mean femur lengths) and heavier than were the intact males.

Discussion. It is difficult to interpret the failure of orchiectomy to reduce the amount of joint disease in STR/1N mice in view of different findings by others already noted. It is conceivable that strain differences have an important role in determining the effect of the testes on osteoarthritis. The apparent arthritis-sparing influence of femaleness in STR/1N mice was not simulated by removal of the testes; presumably an ovarian contribution must be involved in the sex difference.

The increased length of the bones in the castrate mice does not lend support to the view (3) that the growth-promoting effects of testicular or other hormones have an important enhancing effect on degenerative joint disease in mice. Castration prevented the obstructive uropathy already referred to. This lesion was absent from the intact animals that survived for 16 months and so were included in this study. For this reason, any debility attending the uropathy can be excluded as a contributory factor to the bone growth or articular findings. The greater obesity of castrate than intact males probably does not account for the failure of orchiectomy to reduce the amount of joint disease. Not only are the STR/1N females as obese as the males, but other studies (4) on males of several strains, including STR/1N, have shown that obesity *per se* does not have deleterious effects on the joints of mice. The relatively lower body weights in intact males than in females in the present experiment, on the basis of past experience, is the result of a decline from peak values that occurs in this strain in the second year of life. Femur lengths were comparable in intact animals of both sexes. We have found this true also in other strains, although body weight and tail-snout lengths are generally less in female mice than in males.

Summary and Conclusions. Osteoarthritis of the knees was less frequent and severe in 16 month old female than male STR/1N mice. Orchiectomy, performed at 4-6 weeks of age, failed to reduce the amount of joint disease in males. Femur length was increased in the castrate animals.

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Erythropoietic Activity of Tissue Homogenates.* (27070)

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An erythropoietically active factor has been unequivocally demonstrated in the plasma of a wide variety of mammals, including the human, by many investigators. Early attempts to find a similar factor in tissues, using extraction procedures effective for plasma, were not successful(1). Current tissue work employing other extraction methods and assays of increased sensitivity have been more fruitful. Naets(2) has demonstrated erthropoietic activity in saline extracts of dog kidney, liver

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and spleen. Lagrue *et. al.*(3) have found similar activity in phosphate buffer extracts of rabbit kidney. Their preparations of spleen, liver and lung were inactive.

In a preliminary report(4) we indicated that homogenates of muscle, spleen, liver, kidney and brain, prepared from normal and phenylhydrazine anemic rats, were capable of stimulating erythropoiesis. The present communication reports results of extension of these studies.

Materials and methods. Albino rats of a Sprague-Dawley strain were used. Animals serving as tissue donors were retired, female breeders weighing 300 to 350 g. Recipient, experimental animals were females weighing 180 to 220 g. Tissues tested were brain, kidney, liver, muscle and spleen. They were obtained from ether anesthetized donors following aortic exsanquination, and scalpel minced in a petri dish over ice. The mince from 16.0 g of organ was twice washed with 100 ml of modified Tyrode's solution (contained 1% glucose) at 5°C., centrifuged at 3000 r.p.m. in the cold and supernate discarded. The sediment was glass homogenized at 5°C. in a small quantity of Tyrode's solution. Homogenates were prepared fresh daily for injection. In certain instances tissues were frozen in acetone dry ice for future homogenate preparation. Frozen and fresh samples behaved similarly in these experiments. Homogenates were prepared in like fashion from rats rendered anemic with phenylhydrazine. Tissues from these latter animals were obtained when their hematocrits had fallen to between 20 and 25%.

Boiled homogenates, from either normal or anemic donors, were prepared by adjusting the pH of the homogenate to 5.5 with 1 N HCl and boiling for 10 min.

Supernates from homogenates were prepared by centrifugation at 40,000 g for ½ hr in the preparative ultracentrifuge. Concentrated liver supernate was prepared after ultracentrifugation by lyophilization and subsequent dialysis against 1% glucose.

In 2 experiments homogenates were prepared, in a manner similar to that for the rat, from the kidney and liver of normal rhesus monkeys.

All preparations were assayed for erythropoietic activity in dehydrated rats. This is a sensitive assay animal previously described(5), in which has been produced a relative polycythemia with consequent reduction in erythropoietin levels. Water was withheld for 65 hr prior to beginning injection of the test materials. The animals were then injected intraperitoneally daily for 4 days with the materials to be assayed, plus sufficient additional 5% glucose in saline to give 1.5 ml of fluid per 100 g original body weight per day. During the entire period food was allowed *ad libitum*. Homogenates were administered at the rate of 1.0 g (original wet tissue weight) per day. Supernates were calculated to represent 1.0 g original wet tissue weight per day. In the case of concentrated liver supernate, the material was injected in an equivalence of 4.06 g per day. On the fourth day the test animals were given 1.0 μc Fe^{59} I.V. Twenty-four hours later the animals were sacrificed by aortic exsanquination under ether anesthesia. Reticulocyte counts were prepared (dry cresyl-blue method) and incorporation of Fe^{59} into circulating RBC was determined by standard scintillation methods. Control animals received 5% glucose in saline, 1.5 ml/100 g initial body weight per day.

To exclude non-specific effects, groups of test animals were studied following injection of bovine serum albumin (0.2 g/day), gum acacia (25 and 50 mg/day) and methylcellulose (25 and 100 mg/day).

Since RBC retained in the homogenates could conceivably account for erthropoietic activity, quantitative determinations of this residuum were made utilizing Fe^{59} labelled red cells. It was found that a total dose of retained RBC ranging from 0.01 ml to 0.2 ml, depending upon the tissue used, could be expected to be injected into the test animals. Accordingly, groups of assay animals were studied following injection with whole hemolysates of RBC (0.05 and 0.0025 ml/day) and whole, washed RBC (0.05 ml/day).

Results and discussion. The results of the various experiments are summarized in Table I. Tissue homogenates whether from normal or anemic animals produce a consistent and similar erythropoietic response when injected into the rat. This activity is not significantly altered by boiling for 10 minutes at pH 5.5. In centrifuged preparations of normal and ane-

TABLE I. Assay of Erythropoietic Activity.

Material Injected	24 hr Fe^{59} Incorporation in RBC (%)	Reticulocytes (%)	
Brain Homogenate	18.95 ± 7.43*	2.7 ± 1.4*	(11)
Kidney "	15.75 ± 4.87	2.9 ± 1.0	(13)
Liver "	20.83 ± 7.03	2.5 ± 1.5	(15)
Spleen "	20.25 ± 6.65	1.5 ± .6	(3)
Muscle "	17.30 ± 3.43	2.9 ± .8	(8)
Kidney " (Anemic)	21.81 ± 1.62	4.7 ± 2.5	(4)
Liver " "	16.89 ± 6.51	3.2 ± 3.0	(8)
Spleen " "	18.74 ±10.08	2.3 ± .9	(16)
Liver " (Boiled)	13.56 ± 6.76	1.9 ± 1.5	(7)
Muscle " "	17.30 ± 7.95	3.5 ± 1.7	(4)
Brain " "	18.27 ± 6.99	2.1 ± .5	(3)
Kidney " "	14.22 ± 3.66	2.9 ± 1.3	(4)
Spleen " (Anemic Boiled)	15.78 ± 6.96	1.8 ± .9	(10)
Spleen Supernate	9.12 ± 3.22	.9 ± .7	(11)
Spleen " (Anemic)	12.67 ± 3.23	1.4 ± .4	(11)
Spleen Sediment	22.82 ± 8.94	2.1 ± 1.1	(12)
Spleen " (Anemic)	15.90 ± 5.35	2.0 ± .9	(12)
Liver Supernate (Concent.)	15.66 ± 5.12	2.4 ± .4	(4)
Kidney Homog. (Monkey)	11.66 ± 2.21	1.9 ± .7	(4)
Liver " "	13.28 ± 8.02	2.3 ± 1.2	(4)
Bovine Albumin	6.13 ± 2.29	.4 ± .4	(3)
Gum Acacia (25 mg/day)	3.21 ± 1.07	.3 ± .2	(3)
" " (100 mg/day)	4.82 ± .84	.9 ± .2†	(2)
Methylcellulose (25 mg)	5.59 ± 2.10	1.5 ± .9	(3)
" (100 mg)	5.53 ± 4.55	1.3 ± 1.4	(3)
Hemolyzed RBC (.05 ml)	8.44 ± 2.98†	1.0 ± .3	(4)
" " (.0025 ml)	4.69 ± .93	.7 ± .4	(4)
Whole RBC (.05 ml)	4.09 ± .33	.8 ± .2†	(4)
5% Glucose/Saline (Control)	5.65 ± 2.51	.4 ± .3	(114)

* Mean ± S.D.
† statistically significant at P = .05.
Figures in parentheses indicate number of animals in group.
Underlined values statistically significant, P = .01 or less.

mic spleen, activity is found in both supernate and sediment. Supernates of muscle, kidney and liver (not here tabulated) demonstrated little activity. However, when the liver supernate was concentrated 4-fold significant activity was seen.

That the erythropoietic activity of tissue homogenates is not species specific is demonstrated by the erythropoietic response produced by kidney and liver homogenates from monkeys. That the erythropoietic activity is not a non-specific protein or inert material response is suggested by the failure of bovine albumin and gum acacia to engender erythropoietic responses. Methylcellulose while not increasing Fe^{59} incorporation did produce a reticulocytosis. The mechanism here is obscure. The hemolytic phenomenon produced by methylcellulose in rats normally requires a much longer period of injections before it becomes apparent (6).

Whole hemolysates of red cells in a total dose of 0.2 ml appear capable of stimulating erythropoiesis, though the response is small. Equal quantities of intact red cells have no effect. In the homogenate experiments residual red cells in this quantity are found only in spleen preparations. It appears unlikely that the erythropoietic effect of tissue homogenates can be attributed to their red cell content.

TABLE II. Blood Volumes.

Animals	Blood Vol* % Initial Body Wt	Blood Vol % Final Body Wt	RBC Mass % Initial Body Wt	RBC Mass % Final Body Wt	
Muscle Homogenate	3.37 ± .40	5.09 ± .81	1.95 ± .22	2.94 ± .37	(4)
Control	3.27 ± .18	4.80 ± .00	1.92 ± .07	2.83 ± .21	(3)

* Mean ± S.D.

Figures in parentheses indicate number of animals.

However, the effect of red cell hemolysates is of interest, for it may represent a part of a normal feed-back mechanism controlling erythropoiesis.

It is possible that large quantities of injected protein homogenate could cause a relative reduction in red cell mass by increasing plasma volume. This could stimulate erythropoiesis in an animal relatively polycythemic from dehydration. To test this possibility blood volumes were obtained (using standard Fe^{59} labelling techniques) on control dehydrated animals and dehydrated animals receiving 1.0 g muscle homogenate per day. The results presented in Table II indicate that no blood volume shifts occur, and that this factor cannot account for the erythropoietic effects observed.

Lowering of plasma iron levels with resultant increase in plasma Fe^{59} specific activity could account for the Fe^{59} results observed. Naets has demonstrated that this may occur when dog tissue homogenates are injected into the rats(2). From our own results the increase in specific activity would require a reduction in plasma iron levels to 30% or lower of control. As judged from Naets' data this is an unlikely possibility. In addition our Fe^{59} results, as a measure of erythropoiesis, are corroborated by substantial elevations in reticulocyte counts.

The current findings are contrary to previously recorded results(1,7). The past inability of many investigators, including ourselves, to demonstrate erythropoietic activity in tissues is most probably related to the methods of preparation and utilization of less sensitive assay procedures.

Whether the erythropoietic activity of tissue homogenates is mediated by the same principal (erythropoietin) which has been found in plasma is unknown. In both instances the materials retain activity after boiling. Dialysis of the homogenates against 1% glucose causes no loss of activity. Plasma activity is not lost by dialysis against water. If the plasma and tissue factors are the same, it would appear that the distribution is ubiquitous, and that the kidney, as has been suggested(8), is not the only site of erythropoietin production.

Summary. Homogenates of rat liver, kidney, brain, spleen and muscle prepared in modified Tyrode's solution and injected into the rat are capable of stimulating erythropoiesis as measured by Fe^{59} incorporation into red cells and reticulocyte counts. The activity of the homogenates does not appear to be a nonspecific effect. Certain points of similarity are noted between the homogenates and plasma erythropoietin.

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