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The Effect of Methylcellulose on Extrarenal Erythropoietin Production (41862)

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Abstract. The macromolecular polymer, methyl cellulose (MC), is a known hepatosplenomegalic agent which promotes a state of experimental hypersplenism in rats. This is characterized by massive splenomegaly, pancytopenia of the blood elements, medullary hypoplasia, and marked gross and histologic alteration of the liver, kidney, adrenals, and lungs. Massive splenomegaly results from storage of this inert material by splenic macrophages. In the present study, chronic MC administration in rats augmented the hepatic Ep response to hypoxia but did not appreciably affect renal production of Ep. Splenectomy resulted in a decrease in the extrarenal Ep response to hypoxia indicating a possible role of the massively enlarged spleen of these MC-treated rats in extrarenal Ep production. The augmentation of extrarenal Ep elaboration may be attributed to a stimulatory effect of MC on the hepatic and splenic macrophages.

The glycoprotein hormone, erythropoietin (Ep), is acknowledged to be the major regulator of erythropoiesis. In mammals, the kidney serves as the primary site of Ep origin (1, 2). Other sites of Ep production exist; the liver is considered to be the major source of extrarenal Ep (3) in the adult mammal. In contrast, the liver is the chief source of Ep in fetal and neonatal animals. In this regard, bilateral nephrectomy (nephrx) of the fetal or neonatal mammal does not significantly alter the Ep response to hypoxia (4) whereas this is almost completely abolished by hepatectomy (hepx) (5).

Liver regeneration following hepx has been shown to be a potent source of Ep (6, 7) when compared to the normal liver. Hepatic regeneration following liver injury evoked by hepatotoxic agents such as CCl_4 (8, 9) or phenylhydrazine (10) results in significant augmentation of hepatic Ep production when compared with normal or saline-injected animals.

The macromolecular polymer, methyl cellulose (MC), is the methylated ether of cellulose (11). It is composed of polymerized dextrose molecules and is an extremely stable entity (11). Chronic administration of MC induces hypersplenism in rats and mice (11–15). In this hypersplenic state there is massive splenomegaly, pancytopenia of the blood elements, and medullary hypoplasia. There is also gross (organomegaly) and histologic (parenchymal cell destruction) alteration of liver, kidney, adrenals, and lungs (11–15). This condition occurs in part due to the sequestration of MC by the splenic macrophages. Hepatic and renal RES elements also engulf MC, although the size of these organs does not change considerably after MC treatment (11). Splenectomy was found to alleviate the anemia associated with MC-induced splenomegaly (19). The ability of MC to induce splenomegaly is apparently species specific. MC produces this effect in rats and mice (11–15) but fails to cause splenomegaly in dogs (16), rabbits (17), or the rhesus monkey (18). Regardless of its ability to promote splenomegaly, MC administration results in significant hematological consequences in mice (14, 15), rats (11–14), dogs (16), rabbits (17), and monkeys (18). MC induces a moderate hemolytic anemia in the monkey (18), although splenic size was not significantly altered. However, treatment with MC significantly lowers the RBC survival time in primates (18) as well as rodents (20). In addition, lymphocytopenia has been reported in the rhesus monkey (18), neutrophilia in the mouse (21), and thrombocytopenia in the rat (13) after MC administration. Several mechanisms of MC-induced anemia have been hypothesized: (i) a humoral factor was reported in the urine of MC-induced hypersplenic rats which caused

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anemia and thrombocytopenia when given intragastrically to normal rats (22), (ii) splenic sequestration and hemolysis of erythrocytes also have been reported to influence this anemia (13, 20, 23), and (iii) renal failure was postulated as a cause of MC-induced anemia. In this regard, MC administration resulted in renal damage symptomized by proteinuria, hypocholesterolemia, and hypoalbuminemia (24).

An augmentation of renal Ep elaboration was noted in rats after repeated MC administration (25), possibly due to concurrent hemolysis. At present, little information exists concerning the role of MC on extrarenal Ep production. The purpose of the following experiments was to study the effects of chronic MC administration on the extrarenal (splenic and hepatic) Ep response to hypoxia.

Materials and Methods. Female Long–Evans rats, initially weighing 150–200 g (Blue Spruce Farms, Altamont, N.Y.) were used. At the termination of the experiment (4 weeks duration) the rats weighed 275–325 g.

Experimental animals were subjected to 4 weeks of chronic injections (ip) of methyl cellulose (Methocel, Dow Chemical Co., Midland, Mich.) at a dosage of 20 mg/100 g body weight. The stock methyl cellulose solution was prepared at a concentration of 5 mg/ml saline. Saline (0.9%) was used as the control substance for each group. Hematocrits were taken via the tail vein on a biweekly basis.

I. Surgical procedures. The following surgical procedures were employed:

(i) Bilateral nephrectomy (nephrx): Two 1-cm incisions were made on the dorsolateral flanks. The kidneys were localized, externalized, ligated with 3-O suture, and removed.

(ii) Splenectomy (splenx): A 2-cm mediolateral incision was made parallel to the costal margin. The spleen was localized, externalized, ligated with 3-O suture, and extirpated. Organs were weighed following removal. The surgical procedures did not evoke significant blood loss and the survival rate was greater than 95%.

II. Experimental protocols. All experimental groups consisted of five to eight rats with three trials performed per data point. In total, eight experimental groups were studied.

(i) Rats were injected with methyl cellulose daily (20 mg/100 g body wt) for 4 weeks. At the end of the fourth week the rats were nephrx and rendered hypoxic in a hypobaric chamber for 6 hr at 0.4 atmospheres (atm) of air. Immediately following hypoxia the rats were exsanguinated via the abdominal aorta, their serum pooled, and assayed for Ep (2) in the exhypoxic polycythemic mouse.

(ii) Animals were splenx initially and injected with methyl cellulose for 4 weeks. At the termination of this period, the animals were nephrx and rendered hypoxic (0.4 atm/6 hr). They were bled and their serum assayed for Ep.

(iii) After chronic methyl cellulose administration over a 4 week period, rats were splenx, nephrx, and rendered hypoxic (0.4 atm/6 hr). Blood was collected from the abdominal aorta, serum prepared, and assayed for Ep.

(iv) These animals were injected with methyl cellulose over a 4 week period. At the end of this time, they were exposed to hypoxia (0.4 atm/6 hr) and blood was collected and assayed for Ep.

(v–viii) Saline (0.9%) was substituted for methyl cellulose for each of the previous groups tested (Groups 1–4) and served as a control.

To eliminate the possible influence of hematocrit variation in these studies, whole blood from normal donor rats of the same sex and approximate age was infused after Hemo-Set filtration, into animals in Groups 1–3 (immediately prior to nephrx) in quantities which were calculated to achieve a hematocrit of 40%. The actual mean hematocrit after infusion and the resulting Ep values are given in Table III.

III. Ep assay procedure. All blood collected was assayed for Ep using the exhypoxic polycythemic mouse. In this procedure BF-1 virgin female mice (Blue Spruce Farms, Altamont, N.Y.) are subjected to discontinuous hypoxia in a hypobaric chamber for 19 hr a day at 0.4 atm of air for 2 weeks. At the end of these 2 weeks the mice are polycythemic and following a 2-day recovery period are particularly sensitive to Ep since they produce little or no endogenous Ep.

The test material was administered on Day 3 and radioiron on Day 5 posthypoxia. Ra-

dioiron incorporation into newly formed red blood cells was determined on Day 7 posthypoxia and compared to that of standards obtained with the International Reference Preparation for Ep.

IV. Histological procedures. Representative spleen, liver, and kidney samples were excised from each group of rats, weighed, and frozen in liquid nitrogen prior to cryostat sectioning (5 μm). Sections were fixed on a glass slide and stained with hematoxylin-eosin prior to study.

V. Statistics. Ep values are expressed as the mean (IU/ml) ± 1 standard error of the mean for three separate groups consisting of five to eight animals whose sera was pooled (total, 15–24 rats). Levels of significance (*P* values) are determined using Student's *t* test. Hematocrits are indicated as mean values ±1 standard error of the mean for animals comprising each of the eight experimental groups (i.e., 15–24 rats). Hematocrit values for all animals involved in each of the eight experimental and control groups are given: (i) prior to experimental manipulation and (ii) at the conclusion of the experiment just before sacrifice (Table I).

Results. Splenomegaly was evident in the spleens of MC-treated rats. Mean splenic weight of normal rats was 0.63 g (range 0.55 to 0.67) whereas mean splenic weight of MC-treated animals was 2.51 g (range 1.46 to 3.41), a relative size increase of approximately fourfold. White areas, indicative of MC deposition, were also seen on the surfaces of the MC-treated spleens.

Some degree of hepatomegaly was noted in MC-treated rats (mean normal liver weight of 6.39 g as compared to 7.72 g for MC-treated animals). Livers of these rats were more friable than normal livers and small, localized foci of erythropoiesis were observed but these were not as abundant as those seen after partial hepatectomy (37).

Histological study of the spleen revealed an accumulation of MC in the splenic macrophages and a constriction of the sinusoids. Germinal center sizes diminished but no extramedullary erythropoiesis was noted. In the liver, MC accumulated in Kupffer cells, which underwent a corresponding increase in size and number. Some constriction of the hepatic sinusoids was noted and MC-induced hepatocellular destruction was found in several areas adjacent to engorged Kupffer cells. Splenectomy, when performed at the onset of the experiment, accentuated the Kupffer cell involvement in MC-treated rats.

The appearance of the kidneys of MC-treated rats varied. Kidneys were either normal or anisonephric; however, no correlation was found between hepatic Ep production and the gross appearance of the kidneys of MC-treated rats. MC deposition in the glomerular region was associated with localized necrosis of the renal parenchyma but the frequency of these effects were much lower than those observed with the liver and spleen.

At the termination of the experiment, the MC-treated rats displayed a significantly decreased hematocrit (Table I), when compared to saline-treated animals (Groups 1 and 4 vs

TABLE I. MEAN HEMATOCRITS OF MC- AND SALINE-TREATED ANIMALS[a]

	Mean hematocrit at beginning of experiment	Mean hematocrit at end of experiment
Experimental MC groups		
Group 1. MC, nephrx, hypoxia	41.00 ± 3.28	32.75 ± 1.96
Group 2. splenx, MC, nephrx, hypoxia	43.10 ± 3.01	37.00 ± 3.70
Group 3. MC, nephrx + splenx, hypoxia	40.60 ± 2.43	38.00 ± 2.82
Group 4. MC, hypoxia	43.80 ± 3.50	33.00 ± 3.10
Saline control groups		
Group 5. saline, nephrx, hypoxia	39.40 ± 2.10	39.00 ± 2.60
Group 6. splenx, saline, nephrx, hypoxia	40.00 ± 3.20	40.80 ± 3.91
Group 7. saline, nephrx + splenx, hypoxia	42.00 ± 2.80	41.00 ± 4.02
Group 8. saline, hypoxia	40.00 ± 3.15	40.00 ± 3.00

[a] All values are means ± 1 SEM.

Groups 5 and 8, respectively) ($P < 0.05$). Hematocrits of asplenic MC-treated rats, however, were slightly but not significantly lower than their saline-treated counterparts. This finding is consistent with the previously reported role of the spleen in MC-associated anemia.

Extrarenal Ep production after hypoxia was significantly higher in rats after MC injection when compared to saline-injected controls (Table II: Group 1 vs Group 5) ($P < 0.01$). This was approximately 75% of the renal-Ep response after hypoxia (Table II: Group 1 vs Group 4).

MC-treated rats which were splenx and nephrx prior to hypoxia showed an elevation in hepatic Ep production when compared to saline controls (Table II: Group 2 vs Group 6, Group 3 vs Group 7 ($P < 0.01$), but this response was significantly lower than that observed in the MC-treated anephric hypoxic animals (Table II: Group 1) or in unoperated hypoxic rats (Table II: Group 4) with intact spleens ($P < 0.05$). Splenectomy diminished the extrarenal Ep response to hypoxia regardless of whether it was performed prior to MC administration or after chronic exposure to this substance (Table II: Group 1 vs Groups 2 and 3) ($P < 0.05$). Ep titers in rats rendered anemic by MC were not significantly different than those obtained in similar animals whose hematocrits were raised to normal levels via the infusion of whole blood (Table III). This demonstrates that the influence of hematocrit on the Ep response to acute hypoxia in this animal system was minimal.

TABLE II. MEAN SERUM Ep LEVELS[a]

	Ep (IU/ml)
Experimental MC groups[b]	
Group 1. MC, nephrx, hypoxia	0.77 ± 0.03
Group 2. splenx, MC, nephrx, hypoxia	0.54 ± 0.04
Group 3. MC, nephrx + splenx, hypoxia	0.53 ± 0.06
Group 4. MC, hypoxia	1.14 ± 0.14
Saline control groups	
Group 5. saline, nephrx, hypoxia	0.08 ± 0.01
Group 6. splenx, saline, nephrx, hypoxia	0.06 ± 0.01
Group 7. saline, nephrx + splenx, hypoxia	0.07 ± 0.02
Group 8. saline, hypoxia	1.08 ± 0.10

[a] Values are means ± 1 SEM.
[b] All groups were exsanguinated from the abdominal aorta and assayed for Ep at the conclusion of the hypoxic period.

TABLE III. MEAN SERUM Ep LEVELS[a] AND MEAN HEMATOCRITS IN MC-TREATED TRANSFUSED ANIMALS[b]

	Ep (IU/ml)	Hct
Group 1. MC, nephrx, hypoxia	0.71 ± 0.07	40.9
Group 2. splenx, MC, nephrx, hypoxia	0.40 ± 0.09	39.7
Group 3. MC, nephrx + splenx, hypoxia	0.51 ± 0.05	42.1

[a] Values are means ± 1 SEM.
[b] Whole blood was infused immediately prior to nephrx in volumes which were calculated to raise the Hct for rats in Groups 1–3 into the normal range. Hematocrits immediately prior to infusion are given in Table I, last column; those after infusion are included above.

Discussion. Methyl cellulose is a known hepatosplenomegalic agent which produces a state of experimental hypersplenism in rats (11–13). Our histologic and gross findings agree with previously reported effects of MC on the organs studied. The Kupffer cell, part of the reticuloendothelial system (RES) of the liver, was postulated as the hepatic cellular site of Ep origin (6, 26). Recent studies using immunofluorescence and a purified guinea pig anti-rat Ep have indicated that these cells do indeed store and/or synthesize Ep (27). These macrophages have previously been shown to produce other hematopoietic regulators such as colony stimulating factor for granulopoiesis (28) and several prostaglandins (29).

Methyl cellulose-treated rats display RES stimulation despite storage of MC in macrophages of the liver and spleen (30). This compound was shown to elevate phagocytosis of "RE test emulsion" and colloidal carbon by the liver (30). In addition, splenx resulted in an increase in Kupffer cell phagocytosis of colloidal carbon after MC administration.

The altered hepatic blood flow due to the splenomegaly apparently has little effect on either Kupffer cell phagocytic or secretory functions. In this regard, splenectomy, which significantly retards blood flow, had little effect on the hepatic Ep response to hypoxia in the

hepatectomized animal (6). In addition, phenylhydrazine administration, which promotes splenomegaly, actually augments the hepatic Ep response, presumably due to a stimulation of the liver RES.

In the present study, MC treatment augmented the hepatic Ep response to hypoxia but did not appreciably affect renal elaboration of this principle (Table II: Group 1 vs Group 6, Group 4 vs Group 8). Presumably, this is attributable to the previously reported effects of this substance on macrophages (30). The extrarenal response to hypoxia in rats with livers damaged by MC administration (accumulation of MC in Kupffer cells) approximated that in the renal-intact animal (Table II: Group 1 vs Group 4). Kupffer cell cytoplasmic areas and activity have been correlated to the amount of hepatic Ep synthesized or released after hypoxic challenge (31). The increased hepatic Ep response after hypoxia in these MC-treated rats may be associated with macrophage hyperactivity. In addition, hepatic erythropoiesis has been observed after RES activation due to partial hepatectomy (37) and the administration of chemical agents such as glucan (38), phenylhydrazine (39), and MC (19).

Splenx diminished the extrarenal Ep response to hypoxia (Table II: Group 1 vs Groups 2 and 3) ($P < 0.05$) indicating a positive role of the spleen in extrarenal Ep production. MC-activated splenic macrophages may contribute to the increased extrarenal Ep response to hypoxia. The mammalian spleen has been implicated as a possible source of extrarenal Ep (32, 33), although this organ did not appear to contribute to the Ep response to hypoxia in the hepx animal (6). Splenic macrophages also account for most of the erythrophagocytosis in normal animals (34). The sinusoidal macrophages recognize and destroy physically damaged erythrocytes (34), erythrocytes sensitized with metal cations (35), antibody-damaged red cells (36), red blood cells containing inclusions such as Heinz bodies or Howell–Jolly bodies (34), and old erythrocytes so that their iron can be recycled for the production of new hemoglobin (37). Storage and release of iron by these and other RES cells modulating hemoglobin synthesis are recognized as an important function of this system (37).

Chronic administration of MC in rats augmented the production of extrarenal Ep after hypoxia. Most of this was attributed to increased hepatic production of this principle, although splenic Ep elaboration was significant. In contrast to macrophage-rich organs like the liver and spleen, MC had little effect on Ep production in the kidney, which has a relative paucity of these cells. Furthermore, the site of the renal RES (mesangial cells) may preclude any possible direct stimulatory action of MC, due to its ability to occlude the glomerular vasculature (16, 18, 19).

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