system may be susceptible to control for contraceptive purposes. This is not likely to be achieved by altering the hormonal state of the female since capacitation in the rabbit uterus is known to be under hormonal control (6, 12, 13).

Summary. Human seminal plasma contains decapacitation factor (DF) as measured by the ability to render rabbit sperm incapable of fertilizing rabbit ova. The presence of DF suggests that capacitation of sperm is required for fertilization in the human. Compared to several other species human seminal plasma has low DF potency. The DF activity sediments in the ultracentrifuge similar to that of the rabbit.

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Interrelationship of Iron and Manganese Metabolism* (33341)

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A relationship between iron and manganese metabolism has been suggested by the observation of the incorporation of manganese into the porphyrin molecule of red cells and the increased gastrointestinal absorption of manganese in iron deficiency (1, 2). The present studies were performed to determine variations in the metabolism of each of these elements induced by deficiency or overload of the other.

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Methods. Male albino rats (Wistar strain) weighing 200–250 g were used. The animals were fed a standard rat diet (General Biochemicals, Chagrin Falls, Ohio) except that the iron and manganese content was modified from test to test as stated. Iron absorption was measured following a test dose of 0.5 μCi of ferrous-^{59}Fe citrate per 0.25 mg of ferrous sulfate per 0.5 ml of distilled water, administered through a 17-gauge endoesophageal tube to rats fasted for 16 hr. Whole body radioactivity (0.8 MeV $\longrightarrow\infty$) was measured in a small-animal, whole-body liquid scintillation detector (Packard Armac, LaGrange, Illinois), 3 hr and 7 days after dosing to determine the percentage of the test dose absorbed by the rats.

An isolated intestinal loop technique was utilized to measure manganese absorption be-

TABLE I. Effects of Increased Dietary Levels of Manganese and Iron upon Absorption.

Normal diet	Test diet						
After 8 weeks	After 5 weeks			After 23 weeks			
Fe absorp.[b] (%)	Group[a]	HCT. (%)	Fe absorp.[b] (%)	HCT. (%)	Fe absorp. (%)	Serum Fe (μg/100 ml)	Liver Fe (μg/g)
5.9 ± 3.2 (26)	1 (9)	48	11.4 ± 4.5	47	15.3 ± 3.8	258	17
	2 (7)	48	8.3 ± 3.2	47	9.6 ± 2.3	226	47
	3 (7)	48	3.6 ± 2.5	49	3.3 ± 1.8	170	61

[a] 1: 33 mg of $MnCl_2$/g of food; 2: 33 mg of $MnCl_2$ + 800 μg of Fe/g of food; 3: control diet (50.5 μg of Mn and 320 μg of Fe/g of food); number in parentheses refers to number of animals in each group.

[b] Mean ± 1 SD.

cause of the significant hepatobiliary excretion of this element. The rat was anesthetized with sodium pentobarbital and the peritoneal cavity was exposed through a midline incision. A 6-cm closed loop of small intestine just distal to the ligament of Treitz was isolated with umbilical tape. One-half μCi of ^{54}Mn per 1.0 mg of $MnCl_2$ in 0.5 ml of saline was injected into the lumen of the loop and the abdominal wall was sutured together. One hr after the injection the animal was placed in the counter and the total radioactivity was measured. The animal was then killed and the isolated loop was excised. The radioactivity in the remainder of the carcass was counted and the percentage of absorption was calculated.

Total body excretion of ^{59}Fe following the intravenous injection of 1 μCi of ^{59}Fe/0.08 μg of iron as $FeCl_3$ incubated in 0.5 ml of rat plasma was measured by taking serial whole body counts. Manganese excretion was measured in a similar manner following the intravenous injection of 0.5 μCi of carrier free $^{54}MnCl_2$ in 0.5 ml of saline.

Fecal blood loss was estimated by the intravenous injection of 0.5 ml of ^{51}Cr-tagged autologous red cells into the rat and the subsequent radioactivity in stool collections was counted in the small animal counter.

Nonheme iron content of the liver was measured by the method of Brückman and Zondek (3). Serum iron values were determined by the method of Caraway (4).

Forty μCi of $^{54}MnCl_2$ (carrier free) was injected intravenously, in five iron replete rats and in five rats which had been on an iron deficient diet for 8 weeks. Fourteen days later the animals were exsanguinated. The red cells were washed in saline until there was no radioactivity in the supernatant. The red cells from the animals of each experimental group were pooled and the total radioactivity was measured in the small animal counter. Hemin chloride was then crystallized by the method of Labbe and Nishida (5). The heme crystals were weighed and the radioactivity in the sample was determined. Knowing the original hemoglobin concentration of the sample, the theoretical yield of hemin chloride was calculated and thus, the total number of counts associated with heme in the original sample was determined.

Results. Effect of manganese-loaded diet on iron metabolism. Twenty-six rats were placed on a control diet containing 50.5 μg of Mn and 320 μg of iron/g for 8 weeks, at which point a baseline iron absorption test was performed. The mean value in this group was 5.9%. Thereafter, the animals were divided into three groups receiving different diets: (i) standard diet + 33 mg of $MnCl_2$ + 320 μg of Fe/g, (ii) standard diet + 33 mg of $MnCl_2$ and 800 μg of iron/g, and (iii) control diet (standard diet + 50.5 μg of Mn + 320 μg of Fe/g) (Table I). After 5 weeks the iron absorption studies were repeated. The highest absorption (11.4%) was seen in the rats on manganese-loaded diets. Despite the addition of extra iron to the manganese-loaded diet the absorption in this group (8.3%) was still greater than in the control

group (3.6%). There was no significant difference in the hematocrit values. The respective diets were continued for 23 weeks. The hematocrit and iron absorption values remained the same. The animals were then killed. The histology of the liver was normal in all the groups. The mean value for the hepatic iron concentration was lowest (17 μg/g of liver) in the animals on the manganese-loaded diet. The addition of extra iron to the manganese-loaded diet increased the hepatic iron concentration to 47 μg/g but this was still below the mean value of 61 μg/g noted in the controls.

During the eighteenth week each of four rats from groups 1 and 3 received an intravenous injection of autologous ^{51}Cr red cells. The stools from each group were collected during the subsequent 4 days and radioactivity due to ^{51}Cr was measured in the individually pooled samples. The rats on the manganese-loaded diet excreted a total of 135,149 cpm as ^{51}Cr compared to 82,851 cpm in the control group.

Studies were next performed on rats in which the amount of manganese in the diet was varied within physiological limits. The basic diet was the standard rat diet containing 100 μg of iron/g of food to which either 3 or 50.0 μg of Mn/g of food was added. Seven rats were used in each group. After 5 weeks there was no significant difference in iron absorption between the two groups (8.3 ± 2.4 vs 8.3 ± 3.5%). Iron excretion measured by whole body loss of ^{59}Fe activity was the same in both groups.

Effects of altering body iron balance on manganese metabolism. Manganese absorption was measured in three groups of animals containing six animals each: Group 1) iron deficiency was induced by removal of a total of 20 ml of blood over 8 days, (Group 2) iron overload was produced by intramuscular injection of 100 mg of iron dextran in divided doses over 8 days, and (Group 3) iron stores were not altered. All the animals were maintained on the control rat diet. The iron-deficient animals absorbed more manganese (24.4 ± 6.6%) than the controls (12.5 ± 4.0%) while the iron loaded animals absorbed less (5 ± 2.8%). The rate of

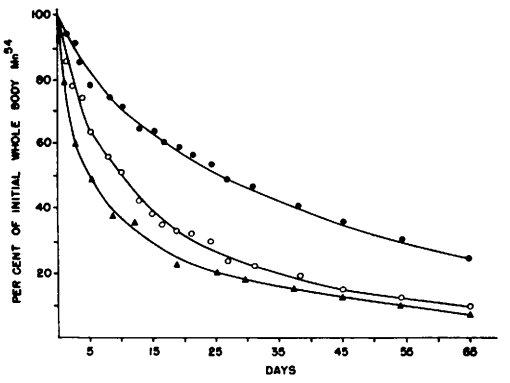

FIG. 1. Whole-body loss of radioactivity following intravenous injection of tracer dose of ^{54}Mn in rats. (●) iron loaded; (○) normal; and (▲) iron deficient.

^{54}Mn excretion in the iron-loaded animals is slower than in the controls whereas the rate of excretion in iron deficient animals is somewhat faster (Fig. 1).

Iron-deficient animals incorporated 0.044% of the injected dose of ^{54}Mn into the peripheral red cell mass. Of the radioactivity in the red cells, 41.1% was crystallized with hemin chloride. In contrast, the normal animals incorporated 0.028% of the injected ^{54}Mn into the red cell mass, and of this 24.9% was crystallized with hemin chloride.

Discussion. Rats fed high doses of manganese had decreased liver iron stores and an increased gastrointestinal absorption of iron. The etiology of this iron deficient state may be related to the demonstrated increase in gastrointestinal blood loss. Large oral doses of manganese chloride may irritate the gastrointestinal mucosa and cause the increased iron loss through bleeding. Mobilization of iron stores and increased gastrointestinal absorption of iron supplied enough iron to provide for a normal red cell mass. However, the presence of elevated serum iron values in this group of animals is not compatible with simple iron deficiency and suggests that the manganese may alter iron metabolism in some other way. Variations in the dietary level of manganese in a physiological range (3–50.5 μg/g) does not alter iron absorption, iron excretion, or hematocrit levels.

Despite large oral doses of manganese for 28 weeks, we were unable to induce hepatic

cirrhosis in the rats which is in contrast to the experience of other investigators (6, 7).

Alterations in iron metabolism also affect manganese metabolism. When iron absorption is increased in iron deficiency, so is manganese absorption increased, and decreased iron absorption in iron-loaded animals is associated with decreased manganese absorption. However, the body appears to compensate for the changes in manganese absorption by increased manganese excretion in the iron-deficient states and decreased manganese excretion in the iron-loaded state. Another interesting observation was the increased incorporation of ^{54}Mn into the porphyrin ring of hemoglobin in iron deficient red cells.

The mechanism of the interrelationship of the metabolism of iron and manganese and the biologic significance remain to be elucidated.

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Dynamics of Luteinizing Hormone (LH) Secretion in the Cycling Ewe: A Radioimmunoassay Study* (33342)

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A marked decrease in the concentration of pituitary LH occurs in the ewe between the fourth and thirty-fifth hours after the onset of estrus, as first shown by Santolucito *et al.* (1) using the ventral prostate bioassay method, and subsequently by Robertson and Hutchinson (2), with the ovarian ascorbic acid depletion (OAAD) assay. Robertson and Rakha (3) later refined these determinations, examining the shorter interval from 12 hr prior to estrus to 10 hr post-onset, and found that a significant decrease from the proestrus level occurred in the first 6 hr post-onset. A 48% decrease in content between the eighth and eighteenth hours post-onset was recently reported by Dierschke and Clegg (4).

It has been assumed that the observed depletions reflected release of the hormone into the circulation in order to induce ovulation. A more direct approach is the determination of circulating levels of LH, which has the additional advantage of allowing multiple sampling of the same animal since sacrifice of the animal is not required. This approach was attempted by Dierschke and Clegg (4), who assayed for LH in the cavernous sinus blood of cycling ewes by the OAAD procedure. They concluded that the serum data provided "only speculative evidence of hormone release". In the present paper we report determination of plasma LH levels in 4 cycling ewes through 2 complete cycles, using a radioimmunoassay technique which we had previously applied to the same samples of sera reported on by Dierschke and Clegg. At that time we found not only much lower values for LH than those reported by them, but also "a general lack of correlation in relative amounts as determined by the two methods" (4).

Materials and Methods. Four Targhee ewes, 30 months of age, all of whom had lambed the previous season, were the experimental subjects during one breeding season

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