cycle(8). In contrast to this, the TP-treated females which were exposed once weekly to males for a 4-week period had a much higher mating percentage for the first 3 weeks. The fact that some of the females became pregnant lowered the number which were sexually receptive during the last week. Pregnancy and unreliable mating behavior are two deterrents against the use of TP-treated females for mating on a once weekly schedule.

Summary. Female rats treated with 10 μg of testosterone propionate (TP) on the 5th day of life had modified estrous cycles through day 72. Alteration of the cycle was predominantly shown by an increased frequency of cornified cells. By day 100 over 80% of the TP-treated females were in persistent vaginal estrus. Placing these females with males for 10 consecutive nights resulted in a sporadic pattern of sexual receptivity. Copulation caused a temporary break in the persistent vaginal cornification. Some of the rats became pregnant, but a high percentage of the pregnancies were terminated by resorption of fetuses. Those females which went to term gave birth to normal appearing young; none of the pregnant females mated after the second exposure. It is concluded that 10 μg of TP given on day 5 will not provide a reliable source of sterile female rats with nymphomania between the ages of 70 and 130 days.

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Iron and Transferrin Distribution and Turnover in Iron-Overloaded Rabbits.* (31060)

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The plasma concentration of transferrin as measured by the total iron binding capacity (TIBC) is found to change in many conditions in man and laboratory animals. In man it rises during pregnancy and in iron deficiency, and it commonly falls in diseases associated with iron overload or with decreased plasma albumin levels. A decrease has also been found in iron-overloaded rabbits(1). Such results, plus the demonstration that transferrin may be bound to the surface of erythropoietic cells(3), have suggested that such binding may occur at the surface of iron-loaded cells of the reticuloendothelial system, and may hence lead to the decreased plasma concentration.

The aim of the present work was to investigate this possibility using iron-overloaded rabbits and radioiodine-labeled purified rabbit transferrin. At the same time the opportunity was taken of determining the effects of iron loading on plasma iron turnover and distribution to the organs of the rabbit, fecal excretion of the injected radioiron, and the presence of any histological evidence of tissue damage due to the excess iron.

Materials and methods. Six female New Zealand rabbits were used. Three of them

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(no. 1-3) were given 26 one-ml injections of iron-dextran (Imferon) over a 5-month period, commencing 4 years before the iron and transferrin turnover studies were carried out. The total dose of iron given was 1300 mg. The other 3 rabbits (no. 4-6), of the same age, received no treatment before the turnover studies, and were used to give control values.

Purification and labeling of transferrin. Rabbit transferrin was purified by ammonium sulfate fractionation, DEAE-cellulose chromatography and zone electrophoresis(6). It was labeled with ^{131}I in the iron saturated form by the iodine monochloride method(5). Freshly obtained rabbit plasma was labeled with ^{59}Fe or ^{55}Fe by the addition of the radioiron as $FeCl_3$ and incubation at 37°C for 30 minutes. The amount of iron added was less than 60% of the latent iron-binding capacity of the plasma.

Procedure for iron and transferrin kinetic studies. The ^{131}I-transferrin and ^{59}Fe-plasma were mixed; aliquots were taken for standards and 2.0 ml volumes were injected into the rabbits by the marginal ear vein. This dose consisted of 2 mg ^{131}I-labeled iron-saturated transferrin containing 50 μc ^{131}I plus 5 μc ^{59}Fe in 1.8 ml plasma. Blood samples were obtained by bleeding into heparinized tubes from the incised marginal vein of the ear opposite to the one injected. The first blood sample (2.0 ml) was taken 5 minutes after the injection while 3 further samples (0.5 ml) were taken during the first hour and other samples (0.5 ml) 3-4 hours after the injection and daily thereafter for 10 days. Hemoglobin, hematocrit, reticulocytes, plasma iron and total iron binding capacity (TIBC) estimations were performed on the first and last samples. Plasma radioactivity for both ^{59}Fe and ^{131}I was determined on all samples. After the injections the rabbits were placed in individual metabolism cages and their urine and feces were collected together daily, homogenized and aliquots counted for ^{59}Fe and ^{131}I. These measurements showed no significant difference between urinary and fecal losses of radioiron and radioiodine in normal and iron-loaded animals. Throughout the experiment the rabbits were fed a standard laboratory rabbit diet, and for 2 days before the injections and thereafter their drinking water contained 0.2 g potassium iodide per liter.

On the 9th day after the initial injections the animals received a second injection of plasma-bound ^{55}Fe containing 50 μc ^{55}Fe in 5 ml pasma. Twenty-four hours later they were exsanguinated by heart puncture under ether anaesthesia. The liver, spleen, kidney, stomach, small intestine, large intestine and bone marrow from both femora were removed, washed where necessary with iron-free physiological saline, weighed, and stored at —20°C until analyzed. They were then homogenized in a Waring blendor and aliquots were taken for non-heme iron and radioactivity (^{59}Fe, ^{55}Fe and ^{131}I) measurements. Precipitation with trichloracetic acid was used to determine how much of the tissue ^{131}I was protein-bound. Small samples were taken from each organ immediately after removal from the animals, fixed in neutral formalin and sections were cut and stained with hemotoxylin-eosin, and with Perls' stain for iron.

Analytical methods. Hemoglobin was determined by the cyanomethemoglobin method, hematocrit by the microhematocrit technique and reticulocytes by staining with new methylene blue. Plasma iron and TIBC(7) and tissue non-heme iron(4) were also measured. Plasma transferrin concentration was estimated from the TIBC value assuming a molecular weight of rabbit transferrin of 90,000. Plasma volume was determined from the plasma ^{131}I value obtained by extrapolation of the concentrations in the first 4 samples back to zero time and from the injected dose.

The plasma and tissue samples and standards were counted for ^{131}I and ^{59}Fe using a gamma radiation detector with a pulse-height analyzer. Iron-59 and -55 were also counted in each sample after wet ashing and electroplating(1). Preliminary experiments showed that ^{131}I was completely lost from the sample during electroplating and therefore did not contaminate the electroplated samples. Plasma iron turnover was calculated from the plasma iron concentration and plasma ^{59}Fe radioactivity values(1) and transferrin distribution and turnover from the plasma and

TABLE I. Tissue Non-Heme Iron Concentrations of Iron-Loaded (1-3) and Control Rabbits (4-6).

Rabbit	Liver		Spleen		Kidney		Stomach		Small intestine		Large intestine		Bone marrow	
	Conc*	Amt†	Conc*	Amt†	Conc*	Amt†	Conc*	Amt†	Conc*	Amt†	Conc*	Amt†	Conc*	Amt†
Iron-loaded														
1	4330	3516	2600	25	198	27	28	7	48	13	31	7	122	3
2	4420	4659	1190	7	264	40	22	5	30	10	35	10	190	3
3	5900	3463	1120	12	259	32	27	6	37	10	21	6	296	6
Normal														
4	116	71	123	.8	39	5	17	3	9	3	22	5	53	.8
5	180	120	981	10	58	9	10	2	23	6	13	3	224	4
6	135	88	213	2	53	8	15	3	20	9	7	2	113	2

* Concentration in mg/100 g wet wt.

† Amount of iron in whole organ.

urine plus feces ^{131}I values(8).

Results. The mean non-heme iron concentration of all the organs examined was higher in the iron-loaded than in the control rabbits (Table I). This difference was most marked in the liver, but was also prominent in the spleen and kidney. By far the greatest amount of the iron excess was in the liver which contained 351, 466 and 341 mg non-heme iron in the 3 treated rabbits compared with 7, 18 and 9 mg for the controls. In contrast, the other organs together contained only 8.3, 7.4 and 8.3 mg in the iron-loaded and 1.6, 3.5, and 2.4 mg in the control rabbits. Histological examination showed marked increases in stainable iron in liver, spleen and kidney of the treated animals, especially in the liver. Here iron deposition was mainly in the perilobular regions, especially along the portal tracts. Closer to the centers of the lobules, there was less stainable iron in parenchymal cells, but the Kupffer cells still contained prominent granules. There was a slight increase of connective tissue around the portal tracts but not elsewhere in the liver. The stainable iron in the kidneys was present as small granules mainly in the cells of the distal parts of the proximal convoluted tubules, but also in much less amounts in the capsular cells of the glomeruli.

The plasma iron concentrations of the iron-loaded rabbits were somewhat lower than those of the controls, while their TIBC values were much lower. As a result of the greater fall of TIBC than of iron level the degree of saturation of transferrin with iron was higher in the treated animals. However, there was no difference between the groups in plasma iron turnover or percentage incorporation of injected ^{59}Fe into erythrocytes (Table II). The decreased plasma TIBC of the iron-loaded rabbits was associated with decreased total body transferrin pool and decreased absolute transferrin turnover rate, but with no change in fractional turnover rate or relative distribution of the protein between extravascular and intravascular spaces (Table III).

The livers of the iron-loaded rabbits contained 10.1, 8.3 and 16.4% of the injected dose of ^{59}Fe and 9.4, 16.0 and 14.7% of the

TABLE II. Body Weights, Hematocrit and Iron Turnover in 3 Iron-Loaded (1-3) and 3 Control Rabbits (4-6).

Rabbit	Body wt (kg)	Hematocrit (%)	Plasma iron concentration (μg/100 ml)	TIBC (μg/100 ml)	Plasma iron turnover (mg/kg/day)	^{59}Fe incorporation into erythrocytes (%)
Iron-loaded						
1	3.95	31.8	220	240	.87	87
2	4.66	37.0	242	247	.82	90
3	3.76	34.9	225	222	.67	82
Normal						
4	3.82	39.6	312	384	.80	85
5	4.32	38.2	297	336	.74	80
6	4.21	39.0	233	326	.75	83

dose of ^{55}Fe, compared with control values of 5.5, 13.1 and 5.1 for ^{59}Fe and 5.6, 4.5 and 4.6 for ^{55}Fe. In the organs other than liver and spleen, there was no difference in radioiron values between control and treated rabbits, but in both groups they contained a greater fraction of the dose of ^{55}Fe than of ^{59}Fe. The livers of the iron-loaded rabbits contained an amount of ^{131}I equivalent to that present in 2.5, 2.0 and 1.5 ml plasma, and of the controls to 1.0, 1.0 and 1.3 ml plasma. There was no difference between the two groups in tissue distribution of ^{131}I in any of the other organs examined. In all organs more than 85% of the ^{131}I was protein-bound.

The amount of ^{59}Fe excreted in urine plus feces was the same for iron-loaded and control animals. The average daily excretion rose during the first 4 days of the experiment to 0.35% of the injected dose and remained there until the end of the experiment.

Discussion. The main differences between treated and control rabbits were in the tissue non-heme iron values and the plasma TIBC. Plasma iron concentrations of the iron-loaded rabbits, rather than being elevated, were somewhat lower than in the controls. This was due to the decreased TIBC which was below the control plasma iron level so that even though the TIBC of the treated rabbits was almost saturated with iron their plasma iron concentration was still below that of the controls.

In the iron-loaded rabbits most of the excess iron was stored in the livers, largely in portal areas. It has been shown that after iron-dextran injections the iron is first deposited mainly in liver parenchymal cells, and over the following months becomes redistributed to the Kupffer cells(2,10). The means whereby this change occurs is unclear, perhaps phagocytosis of iron-containing parenchymal cells by reticuloendothelial cells. The failure to demonstrate any real evidence of liver cirrhosis despite iron loading of portal areas even after 4 years of severe iron overload is consistent with the results of many other investigators who have failed to produce cirrhosis and the pathological picture of hemochromatosis in experimental animals by the use of iron overload alone(1).

The high transferrin saturation may be the reason for the greater uptake of radioiron by the liver in the iron-loaded animals than in the controls. It has been found that during *in vitro* incubation iron uptake by liver tissue increases as the degree of saturation of transferrin with iron is increased(9).

The low TIBC values of the iron-loaded rabbits were associated with decreased total body transferrin pools, but unchanged transferrin extracellular:intravascular distribution ratios when compared with the controls. Actual measurement of tissue ^{131}I showed the livers of the treated animals to contain a little more ^{131}I than in the controls. The difference was, however, equivalent to only about 1 ml plasma, less than one percent of the plasma volume. The decreased plasma transferrin concentrations associated with iron overload were therefore not due to extravascular sequestration of the protein, but to decreased rates of synthesis as shown by the absolute transferrin values (Table III). Possibly increased non-heme iron in the transferrin synthesizing cells impairs their synthesis of the

TABLE III. Transferrin Distribution and Turnover in 3 Iron-Loaded (1-3) and 3 Control Rabbits (4-6).

Rabbit	Plasma vol (ml/kg)	Total body transferrin (mg/kg)	Transferrin extravascular: intravascular ratio	Fractional transferrin turnover (plasma pools/day)	Absolute transferrin turnover (mg/kg/day)
Iron-loaded					
1	32.9	148	1.34	.35	22.0
2	26.0	137	1.62	.40	20.3
3	27.9	120	1.43	.34	16.9
Normal					
4	24.9	198	1.60	.36	27.6
5	28.1	188	1.49	.35	26.2
6	26.2	183	1.68	.36	24.3

protein. The actual cells involved have not been established.

Summary. Iron overload was produced in 3 rabbits by intramuscular injection of 1,300 mg iron as iron-dextran, while 3 control rabbits received no treatment. Four years later, plasma iron and transferrin turnover and distribution in the main abdominal organs were studied using plasma-bound radioiron and ^{131}I-labeled rabbit transferrin. The iron-labeled rabbits when compared with the controls had increased non-heme iron concentrations in liver, spleen, kidney, stomach and intestine; most of the excess iron was in the livers which showed only slight increases of connective tissue around the portal tracts where most of the stainable iron was deposited. Iron-loading was associated with decreased plasma transferrin concentration and total body transferrin and decreased absolute turnover of transferrin. The distribution of transferrin between extravascular and intravascular spaces was the same in both groups of rabbits. Hence the decreased plasma transferrin level in the iron-loaded animals was probably due to lowered rates of synthesis.

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Absorption of 14C Triolein in the Bile Fistula Rat. (31061)

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Previous *in vitro* experiments(1) demonstrated that conjugated bile salts facilitate the esterification of fatty acids by rat small intestinal mucosa. Furthermore, analyses of thoracic duct lymph and portal blood *in vivo* (2,3) suggested that there was a decreased esterification of absorbed fatty acids by the small bowel mucosa of bile fistula rats. This possibly accounted for the portal transport

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