demonstrated that absorption is enhanced in iron deficiency and depressed in iron overload(14). The failure of phytate and desferrioxamine, chelaters of dissociated iron, to reduce the absorption of heme iron as it does the salt has led others to suggest that in man there are two different pathways for the absorption of iron, one for heme, the other for ionized iron. The results of our experiments could mean that the rat lacks the first pathway. Such an observation must reinforce our caution in interpreting the results of experiments on rats in terms of human physiology.

Our finding that hemoglobin iron is poorly absorbed by rats even when they are iron deficient is in conflict with Bannerman's recent report that hemoglobin iron is well absorbed by rats(15). The reason for the discrepancy is not evident.

Summary. In the rat the absorption of a test dose of hemoglobin or hemin iron^{59} was significantly less than a comparable dose of iron^{59} as $FeSO_4$. That this is of physiologic significance was demonstrated by producing a state of iron deficiency in animals raised on a diet with hemoglobin as the only source of iron. These findings are in contrast to recent observations in man and suggest a difference between species as well as a difference in the epithelial cells' ability to sequester iron as the salt or in the heme ring.

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Phagocytosis of Heterologous Red Cells in Mice Injected with Isologous Microsomes.* (30671)

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Earlier findings of increased reticuloendothelial (r.e.) phagocytosis during restoration of liver tissue after partial hepatectomy(1) and changes observed in the phagocytic activity of tumor-bearing rats(2) and mice(3) furnished the incentive for investigating phagocytosis in mice after administration of subcellular tissue fractions. This approach was expected to provide some insight into cellular events associated with processes of physiologic or pathologic tissue breakdown, and subsequent repair and regeneration. In planning these experiments, it was essential to exclude, as much as possible, interfering factors related to isoimmunization, *i.e.*, introduc-

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tion into the recipient of tissues differing in isoantigenic composition from his own. Hence, we used either inbred mice, or F_1 hybrids derived from 2 inbred strains. To avoid immunization to the Y-linked male histocompatibility antigen discovered by Eichwald and Silmser(4), recipients and tissue donors were either of the same sex, or female donors were used for female and male recipients. Preliminary results have been reported(5).

Materials and methods. Animals. Mice, 12 to 24 weeks old, were either derived from strains C3H/S, AKR/S, and C57BL/S, bred in our laboratory by brother-to-sister mating, or were F_1 hybrids of the following parentage: C57BL/S × C3H/S; C57BL/S × A/Sn/S; C57BL/S × BALB/c/S; DBA/S × BALB/c/S.

Tissue fractions. A suitable number of donor mice of the same genetic background as the prospective recipients were killed, the organs to be used (liver, spleen, kidney) immediately removed, weighed, and placed into crushed ice. After addition of 10 ml of ice-cold 0.25 M sucrose solution per g of tissue, the mixture was homogenized in a Potter-Elvehjem homogenizer. The homogenate was subjected to differential centrifugation, modified from conventional methods(6,7), in a Spinco refrigerated centrifuge (model L, rotor no. 40). The usual procedure consisted of a first centrifugation for 10 minutes at 12,000 *g*, the supernatant of which was saved. The sediment of this centrifugation was mixed with half the initial amount of sucrose solution used, rehomogenized, and subjected to a second centrifugation similar to the first in order to sediment nuclei and mitochondria. The combined supernatant fluids of the first 2 centrifugations were centrifuged for one hour at 105,000 *g*, leading to sedimentation of the microsomal fraction. In some experiments, nuclear and mitochondrial fractions were prepared separately. For injections, the particular fractions were used as 10% suspensions in saline. The tissue homogenates or fractions were either used within a few hours of preparation, or preserved at —20°C for subsequent use. Determinations of nitrogen (Kjeldahl) and, in the later part of the study, of RNA [orcinol method(8)] were done on aliquots of the homogenates and fractions.

Basic experimental design. On 3 successive days, 0.2 ml of the tissue suspension was injected intravenously or intraperitoneally into the experimental mice, while suitably matched control animals received injections of 0.2 ml of saline. Approximately 6 hours after the last injections, all mice were injected intraperitoneally with Cr^{51}-labeled sheep red cells.

Assay of phagocytosis. Within 3 hours of administration, sheep red cells were labeled with Cr^{51}‡ according to procedures previously described(9). The sheep red cells were suspended in saline in a concentration of 10%. Each mouse was injected with 0.5 ml of this suspension, the radioactivity of which corresponded to 0.2 to 0.5 μc. The mice were killed 24 hours after the injection of red cells, the livers and spleens removed, weighed, and subjected to radioassay in a scintillation well counter.§ The percentage of the injected radioactivity recovered in liver and spleen was calculated and converted into per cent of uptake per g of liver and per 100 mg of spleen.

Analysis of results. Mean values and standard deviations of hepatic and splenic uptakes of radioactivity were determined for control and experimental groups, and served as indicators of phagocytic activity. Differences observed between control and experimental groups were tested for statistical significance by means of Student's *t* test, or by means of a test for multiple comparisons(10) when the results of more than one experimental group were compared with those of the control group.

Results. Fig. 1 illustrates an experiment in which the microsomal fraction prepared from isologous liver was injected into (C57BL × C3H)F_1 mice, either intravenously or intraperitoneally, prior to injection into experimental and control mice of Cr^{51}-labeled sheep red cells. There was a pronounced depression of hepatic and splenic phagocytosis in experimental mice, independent of sex of the

‡ Squibb Chromitope Sodium, 100 uc/ml.

§ RIDL Scintillation counter, model 60-2; 11-1 well with 1¾ x 2″ crystal.

TABLE I. Effect of Microsomal Fractions on Phagocytosis of Sheep Red Cells.

Exp No.	Strain	Sex	Group*	No. of mice	% injected CPM per ± S.D. g of liver	% injected CPM per ± S.D. 100 mg of spleen
1	AKR	♂	C	10	24.4 ± 2.9	3.3 ± 2.1
			M-L	7	6.5 ± 3.7†	.7 ± .5†
2	C57BL	♀	C	6	28.2 ± 3.8	8.2 ± 1.3
			M-L	6	12.2 ± 2.2†	3.1 ± 1.2†
3	(DBA × BALB/c)F_1	♂	C	10	25.1 ± 6.6	4.1 ± 1.7
			M-L	10	9.3 ± 4.0†	1.9 ± 1.0†
4	(C57BL × A/Sn)F_1	♂	C	5	26.3 ± 7.8	6.9 ± 3.6
			M-L	5	11.4 ± 4.4†	2.9 ± 2.4
5	(C57BL × A/Sn)F_1	♀	C	7	23.4 ± 5.6	9.0 ± 3.5
			M-L	7	10.7 ± 3.2†	3.5 ± 1.4†
			M-K	7	9.0 ± 4.8†	2.8 ± 1.5†
6	(C57BL × BALB/c)F_1	♂	C	4	16.5 ± 3.1	4.7 ± 2.2
			M-L	4	7.6 ± 2.9†	1.8 ± .2†
			M-K	4	5.4 ± 3.7†	1.8 ± 1.0†
			M-S	4	4.7 ± 2.5†	1.6 ± .7†

* C = Control; M = Microsomal fraction; L = Liver; K = Kidney; S = Spleen

† Comparison between experimental and control groups: $P < .01$.

animal and route of administration. The differences between mean values of uptakes determined for control and experimental groups were significant at the 0.01 level.

Table I summarizes 6 additional experiments in which the phagocytosis of red cells was compared in control animals and in mice treated with intraperitoneal injections of microsomes. Consistent and significant depressions of hepatic and splenic phagocytosis resulted from administration of microsomes to mice of Experiments 1 through 4, with only Exp. 4 failing to show a statistically significant difference for splenic phagocytosis. Exp. 5 and 6 included groups of mice injected with microsomal fractions prepared from isologous kidney or spleen. These tissue fractions produced effects indistinguishable from those of liver microsomes. The tissue suspensions of 5% concentration used in Exp. 6 reduced phagocytic uptake to about the same extent as did the 10% suspensions used in the other experiments.

Results of experiments comparing the effect on subsequent phagocytosis of red cells of unfractionated homogenate of isologous liver, of the microsomal fraction, and of the supernatant removed after sedimentation of microsomes, are shown in Table II. In additional experiments, not recorded, nuclear and mitochondrial fractions were also tested in parallel with microsomes. In all experiments, administration of microsomal fractions depressed the uptake of subsequently injected sheep red cells most consistently and most effectively.

Table III presents results of one of several experiments designed to determine the duration of the interference with phagocytic activity resulting from treatment with microsomes. The decrease of hepatic as well as

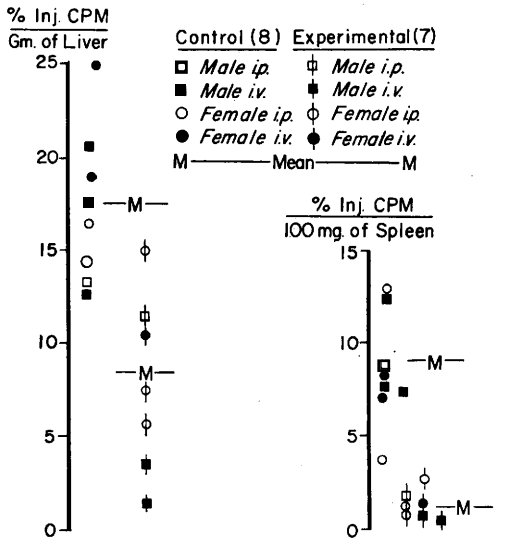

FIG. 1. Effect of injection of liver microsomal fraction on phagocytosis of sheep red cells in (C57BL × C3H)F_1 mice.

TABLE II. Effect of Isologous Liver Fractions on Phagocytosis of Sheep Red Cells.

Exp No.	Strain	Sex	Group*	No. of mice	% injected CPM per ± S.D. g of liver	% injected CPM per ± S.D. 100 mg of spleen
1	(C57BL × A/Sn)F_1	♂	C	4	14.0 ± .8	4.3 ± 3.2
			H	4	13.0 ± 2.5	2.0 ± .5
			M	4	5.4 ± 1.8†	1.1 ± .5†
			S	4	12.6 ± 3.6	2.9 ± .9
2	AKR	♂	C	8	30.2 ± 7.7	3.7 ± 1.2
			H	9	11.9 ± 5.7†	2.2 ± 1.7
			M	9	7.0 ± 5.0†	.9 ± .5†
3	C3H	♀	C	5	20.6 ± 3.8	5.2 ± 2.9
			M	4	3.7 ± 1.2†	.5 ± .1†
			S	5	15.7 ± 3.2†	1.8 ± .7†

* C = Control; H = Homogenate; M = Microsomes; S = Supernate
† Comparison between experimental and control groups: P <.01.

splenic phagocytosis was most marked when the labeled sheep red cells were administered 6 hours after the last of 3 intraperitoneal injections of microsomes. When the red cells were given 24 hours later, the difference between control and experimental mice was less pronounced, though statistically significant. After extension of the interval between the last injection of microsomes and red cells to 48 hours, the depression of hepatic phagocytosis was not significant, and that of splenic phagocytosis was of borderline statistical significance.

To test the stability of microsomal fractions, a suspension prepared from isologous liver was divided into 3 portions: the first was given to mice within the first 3 days of preparation, in accord with the procedure used in the experiments described; the second portion was stored at 4°C for 7 days before injection into the second group of mice, the third portion was subjected to repeated freezing and thawing during a 7-day period prior to use for the third group of mice. Treatment of mice with either of the 3 microsome portions reduced the uptake of subsequently injected sheep red cells, as compared with the findings in simultaneously tested saline-injected control mice, the frozen-thawed portion of microsomes being less effective than the other portions. In a further experiment, injections of microsomal fraction given on each of 3 successive days were followed by a rest period of 48 hours, after which 2 more injections were given on successive days. This treatment produced the same decrease in uptake of labeled sheep red cells as did the "standard" procedure used in the other experiments. No untoward effects on the health of the animals were observed in any of the experiments as a result of treatment with isologous homogenates or subcellular fractions, with the exception of some deaths of mice injected intravenously, presumably caused by embolism with coarse particles present in the suspension. Autopsies, routinely performed at the end of the experiments, did not reveal any gross abnormalities, except for slight to moderate hepatomegaly and splenomegaly encountered in some experimental mice. There was no correlation between the changes in size of liver and spleen and the uptake of red cells in these organs. Histologic studies, done in some groups, likewise failed to reveal any significant deviations from the normal.

TABLE III. Duration of Depression in Phagocytosis in Female AKR Mice Injected with Hepatic Microsomes.

Interval between test and last injection of microsomes (hr)	Group*	No. of mice	% injected CPM ± S.D. g of liver	% injected CPM ± S.D. 100 mg of spleen
6	C	2	39.7 ± 9.8	3.6 ± .5
	M	3	7.0 ± 1.6†	.7 ± .1†
24	C	3	38.5 ± 1.7	3.6 ± .3
	M	3	19.9 ± 3.2†	1.4 ± .1†
48	C	3	35.0 ± 3.5	5.9 ± .4
	M	3	26.2 ± 7.3	3.2 ± 1.4‡

* C = Control
M = Microsomes
† P <.01
‡ P <.05 >.01

TABLE IV. Phagocytosis of Sheep Red Cells After Prolonged Administration of Isologous Liver Microsomes (DBA × BALB/c)F_1 Male Mice.

Treatment		Group*	No. of mice	Liver		Spleen	
				% body wt	% inj CPM ± S.D. (g)	% body wt	% inj CPM ± S.D. (100 mg)
No. of wks	inj						
3	10	C	5	4.7	20.7 ± 6.3	.5	1.8 ± .9
		E	5	4.8	10.2 ± 7.4†	.5	1.0 ± .6
7	21	C	5	5.2	34.3 ± 9.3	.4	2.7 ± .5
		E	5	6.0	20.6 ± 12.0	.7	1.6 ± .5
11	33	C	3	5.4	32.7 ± 5.2	.5	2.6 ± .2
		E	5	6.9	3.6 ± 2.2‡	.8	.4 ± .2‡

* C = Control
E = Experimental

† P <.05 >.01
‡ P <.01

Table IV summarizes findings obtained in male (DBA × BALB/c)F_1 mice, 4 to 6 months old at the start of the experiment, and subjected to administration of isologous liver microsomes over extended periods of time. Mice of the experimental group received 3 weekly intraperitoneal injections of 0.2 ml each of the 10% microsome suspension, while mice of the control group were injected with corresponding amounts of saline. When labeled sheep red cells were injected after 3 weeks of treatment into control and experimental mice of the first group, a moderate reduction of red cell phagocytosis was observed in experimental mice. In the second group, tested after 7 weeks of treatment, experimental mice showed an even lesser degree of inhibition of phagocytosis, but the phagocytic activity of microsome-treated mice of the final group, tested at the end of 11 weeks, was severely depressed. Additional groups of mice, not included in the Table, were injected with isologous splenic microsomes and showed changes in phagocytic activity comparable to those observed in mice treated with liver microsomes. Hepatomegaly and splenomegaly, noted in some short-term experiments, were consistently observed in mice undergoing prolonged treatment with microsomes. Nevertheless, experimental mice showed no impairment of general health nor were other gross abnormalities detectable at autopsy.

Some data on chemical composition of the tissue homogenates and fractions, as used for injections, are found in Table V. Although expectedly the nitrogen and RNA contents of individual preparations showed some fluctuations, the N content was consistently higher in nonfractionated homogenates than in microsomal suspensions. On the other hand, microsomal RNA exceeded that of the homogenates from which they were derived.

Discussion. Phagocytic uptake of heter-

TABLE V. Nitrogen and RNA Contents of Tissue Suspensions.

Exp	Organ	mg N/ml*			μg RNA/mg N*		
		Ho	Mi	Su	Ho	Mi	Su
Table 1, #3	Liver	3.92	2.07	.76	—	—	—
" 1, #5	"	3.20	1.69	.75	—	—	—
	Kidney	3.05	1.22	.39	—	—	—
" 1, #6	Liver†	2.62	1.98	.54	53	160	94
	Kidney†	3.19	1.88	.74	—	87	—
	Spleen†	2.34	1.60	.43	43	148	—
" 2, #1	Liver	3.80	2.06	1.02	76	200	—
" 2, #3	"	2.86	1.96	1.58	—	—	—
" 4	" ‡	3.74	2.37	—	107	227	—

* Ho = Homogenate
Mi = Microsomes
Su = Supernate

† 5% suspensions.
‡ Avg values of 4 preparations.
— = not determined.

ologous red cells in mice was significantly and consistently reduced up to approximately 48 hours after parenteral administration of isologous tissue fractions. Since no attempt was made to obtain subcellular fractions of maximal purity, this may account for some variations in the relative activity of unfractionated homogenates and their fractions. Nevertheless, the degree of interference with red cell phagocytosis resulting from use of microsomal fractions, regularly exceeded that of whole homogenate and other subcellular fractions. Microsomal preparations preserved at 4°C retained their biologic activity for one week. Comparison of the N and RNA contents determined in homogenates and fractions makes it unlikely that protein or other nitrogenous compounds are the carrier of biologic activity, as defined in our experimental design; on the other hand, RNA which is present in highest concentration in microsomes may be a good candidate for this role. Additional studies, including treatment of microsomes with Na deoxycholate, and exposure of the substrates to proteases or nucleases, prior to experimental use, may provide some more conclusive information.

Administration of hepatic, splenic, and renal microsomes appeared to be equally effective in depressing red cell phagocytosis. Neither short-term nor prolonged treatment with isologous tissue fractions produced any harmful effects on the health of the animals. The only gross change observed was moderate increase in size of liver and spleen in some mice treated for short periods, and in most animals subjected to extended treatment. In short-term experiments, mice responded to administration of microsomes given 48 hours after a first series of microsome injections, in the same manner as did mice treated for the first time. On the other hand, experimental data obtained with prolonged administration of microsomes suggest that this treatment induces a "waning and waxing" inhibition of phagocytosis. The mechanism responsible for the interference with uptake of red cells in the experiments described is as yet in doubt. Considering a possible competitive phagocytic uptake of the injected substrates by r.e. cells, we attempted to study the localization in liver and spleen of isologous tissue homogenates and microsomes labeled *in vitro* with Cr^{51} prior to injection. Although these experiments failed to show a preferential uptake of the radiolabel in liver and spleen, these findings do not definitely exclude phagocytic deposition of the tissue fractions, but they may rather indicate that the protein-bound Cr^{51} label is not suitable for tracing the fate of the substrates under study.

The evidence pointing to the predominant role played by microsomes in the phenomena presented here, is of particular interest because microsomes, the active sites of protein synthesis, can be assumed to be involved in cellular growth and proliferation. Within the last years, several groups of investigators(11-14) have demonstrated a significant association of H-2 histocompatibility antigens with microsomal fractions of mouse tissues. While the isologous derivation of the fractions employed in our work should eliminate isoantigenic differences as accounting for the findings, it should be noted that Congdon(15) observed, in mice injected with homogenate of isologous liver, regressive and progressive changes in the spleen similar to those encountered after antigenic stimulation. Thus Congdon's as well as our observations suggest that subcellular components may affect lymphoreticular tissues even independent of isoantigenic differences.

In reference to the possible physiologic significance of our findings, the following quantitative factors are of interest: 0.2 ml of the microsome suspension used contained 20 mg of substrate, representing from 60 to 100 mg of fresh liver. Thus the treatment, consisting of 3 such injections, was equivalent to administration of 15 to 20% of the wet weight of the liver of an adult mouse. Whether such material released *in vivo* would produce similar effects and whether these changes play a role in tissue restoration, is at present merely a matter of speculation. In this context it may be noted that inbred rats, injected with isologous liver microsomes, exhibited a similar depression of phagocytosis of subsequently administered sheep red cells as reported for mice, and thus the phenomenon has been observed in two species. On

the other hand, hepatic and splenic uptake of chromic radiophosphate, injected intravenously or intraperitoneally into mice treated with isologous microsomes, did not differ from the uptake in control mice. This confirms the critical importance of the test substrate used for assay of phagocytic function. More definitive interpretation of the changes in r.e. phagocytosis observed in our study may have to await completion of work currently under way in which suggestive evidence has been obtained that microsomes prepared from isologous mouse tumors or from regenerating isologous mouse liver tissue at specific times after partial hepatectomy, do not possess the ability of interfering with red cell phagocytosis to the same extent as do microsomes prepared from intact, normal isologous tissues.

Summary. 1. Parenteral administration to mice of homogenates, and particularly of microsomal fractions, prepared from isologous liver, spleen, or kidney, caused a significant reduction in hepatic and splenic uptake of subsequently injected Cr^{51}-labeled sheep red cells. 2. These changes in r.e. phagocytosis were transient, but they were also observed at certain stages of long-term administration of the tissue fractions. 3. The biologic activity did not appear to be related to the N content of the substrates, but rather to the RNA content. 4. No untoward effects on the health of the mice were encountered, and the only gross changes attributable to the treatment were moderate increases in size of liver and spleen. 5. The possible significance of the changes in r.e. phagocytosis and their relationship to previous work have been discussed.

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Lipid Composition of the Non-Gravid and Gravid Rabbit Endometrium.* (30672)

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Morphological and histochemical studies have revealed changes in the concentration and distribution of endometrial lipids in different stages of the estrous cycle in several species of animals. Nicol and Snell(1) showed that in guinea pigs increasing activity of the corpus luteum was associated with an increased accumulation of endometrial lipid. Ovariectomy caused a disappearance of this lipid, suggesting a regulation of the lipid metabolism of the uterus by the ovarian hor-

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