representing the greater degrees of lysis. It is also possible to employ a correction for unlysed primary cells derived from data such as given in Fig. 1.

Whereas Taliaferro *et al*(1) reported no difference between the slopes of the hemolysin and transfer test von Krogh plots for a given serum, a significant difference was frequently observed in the present studies.

Although a transfer test is useful for indicating qualitative differences between hemolytic antisera, results must be interpreted cautiously. Two antisera with comparable titers and transfer values are not necessarily identical because the proportion and avidity of each hemolysin species which may comprise an antiserum are unknown. Moreover, since fixed transfer and hemolysis times are used in this procedure, the results are dependent on the rates of transfer and hemolysis.

It is reasonable to assume that a similar approach could be used to study antibody transfer in other immune cytolytic systems in which it would be preferable to avoid labeling the target cells.

Summary. A modified method is described for measurement of the avidity of rabbit anti-sheep hemolysin in terms of the proportional transfer of the antibody from one population of erythrocytes to another. It is simple, involves colorimetric measurements only, eliminates the need for pretreatment (*e.g.*, Cr^{51}-labeling) of one of the 2 cell populations, yet yields results comparable to those obtained using an isotopic label.

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Delayed Implantation in Gonadotropin-Treated Immature Rats.* (31410)

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In the study of implantation processes in the rat, a convenient means of obtaining as many blastocysts as possible from a delayed-implantation rat became of importance to us. The fact that the immature rat can be induced to superovulate with pregnant mare's serum (PMS)(1) and, if mated, shows an unusually large number of implantation sites ("superfecundity")(2) encouraged us to produce delayed implantation by ovariectomy and progesterone treatment(3) in the PMS-treated and mated immature rat. Recently, Strauss and Meyer(4) and Strauss(5) in this laboratory developed a regimen for producing ovulation, and high incidence of mating and pregnancy in the prepuberal rat using PMS. We used this basic method to induce superovulation and mating in immature rats, and to obtain large numbers of preimplantation blastocysts and blastocysts which had been prevented from implanting. This was accomplished by injecting human chorionic gonadotropin (HCG) in addition to PMS. The data obtained are the subject of this report.

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Methods. Immature female rats, 26 days old, weighing 65 to 70 g, were obtained from the Holtzman Co., Madison, Wis. They were maintained *ad libitum* on Rockland rat chow and tap water. The room temperature was kept between 74 and 80°F. The light was

TABLE I. Effect of Various Amounts of PMS and HCG on Number of Implantation Sites in Intact Rats.

PMS (IU)	HCG (IU)	No. rats mated	No. rats with impl. sites on P_9*	Total sites	Avg sites /preg. rat ± S.E.
8	.4	3	3	19	6.3 ± .9
12	.4	2	2	16	8.0 ± 1.4
16	0	3	3	62	20.7 ± 5.3
	.4	5	5	98	19.6 ± 2.3
	.6	8	8	144	18.0 ± 3.2
	.8	3	3	48	16.0 ± 4.3
	1.2	6	5	91	18.2 ± 3.2
20	0	6	3	38	12.7 ± 5.8
	.4	3	2	39	19.5 ± 3.5
	.6	8	8	144	18.0 ± 1.8
	.8	5	3	85	28.3 ± 3.2
	1.2	6	5	146	29.2 ± .7
	2.4	3	2	17	8.5 ± 2.0
24	0	7	3	79	26.3 ± .9
	.6	11	5	111	22.2 ± 5.2
	1.2	7	5	94	18.8 ± 5.1

* P_9—Day 9 of pregnancy.

turned on at 5 AM and off at 7 PM Central Standard Time. All times mentioned are colony time(6); midnight is the midpoint of the dark period, noon is the midpoint of the light period.

The rats received various doses of PMS† subcutaneously on Day 30 between 8 and 10 AM, and different doses of HCG, *via* tail vein, on Day 32 between 1 and 2 PM. They were then examined for vaginal opening. If the vagina was not fully open, a small blunt forceps was used to complete the opening and the females were immediately caged with males at a ratio of 2 females to 3 males and examined for vaginal plug or presence of sperm in the vaginal smear on the next morning (Day 33); this was designated as P_1 (Day 1 of pregnancy). Autopsy was performed on P_9 and the implantation sites of the intact rats were counted. It was found that 20 International Units (IU) of PMS followed by 1.2 IU HCG gave the greatest number of implantation sites; this regimen was chosen for the delayed implantation study.

In the delayed implantation study, ovariectomy was performed under ether anesthesia on P_3, care being taken not to injure the oviduct. The rats were injected subcutaneously (s.c.) with 2 mg progesterone in 0.1 ml corn oil beginning with P_3. Implantation was induced in these rats by a single s.c. injection of 1 μg estrone in 0.1 ml corn oil on either P_7, P_9, P_{11}, or P_{16} and implantation sites were counted 2 or 4 days later. The preimplantation blastocysts were flushed from the uterus on P_4 or P_5 and the delayed blastocysts on P_7, P_8, P_9 or P_{13} with saline and counted with the binocular microscope. The oviducts of rats autopsied on or before P_4 were dissected out and pressed between 2 slides and the number of ova counted with the aid of a low power microscope.

Results. Table I shows that as many as 29 implantation sites were obtained with 20 IU PMS plus 1.2 IU HCG. Addition of different doses of HCG to 16 IU PMS did not affect the average number of sites as compared to PMS only. However, when 20 IU PMS was given with various doses of HCG up to 1.2 IU, the greater the amount of HCG the greater the number of sites. At 24 IU PMS, the number of sites tended to be inversely related to the dosage of HCG. Although it has been reported(7) that 33 represents the maximum number of implantation sites, we did observe 2 rats each with 34 implantation sites.

Data from preliminary experiments showed that blastocyst implantation could be delayed in rats superovulated with 20 IU PMS followed by 1.2 IU HCG, but the average number of blastocysts recovered was not so great as expected. Therefore, we made a comparison of the recovery of ova or blastocysts in intact rats with those in ovariectomized rats on various days. The results are presented in Table II. The numbers of blastocysts recovered from the intact and from the ovariectomized rats were 28.0 and 19.2 on P_4, and 23.5 and 20.5 on P_5 respectively. There was no statistically significant difference between these comparable P_4 and P_5 groups, but the difference between the number of sites in the intact (24.5) and the number of blastocysts in the ovariectomized rat (13.0) on P_9 was significant. However, the numbers of induced implantation sites in Group 12 on $P_{11\text{-}15}$ (17.3), and in Group 14 on P_{19} (18.6) and the blastocysts recovered in Group

†"Gonadogen," Upjohn Co., Kalamazoo, Mich.

TABLE II. Numbers of Ova, Blastocysts and Implantation Sites in Intact and Ovariectomized Immature Rats Superovulated with 20 IU PMS plus 1.2 IU HCG.

Group	No. rats	Treatment	Day of autopsy	Avg ova or blastocyst ± S.E.	Avg sites ± S.E.
1	10	intact	P_1*	39.9 ± 3.1	
2	5	"	P_3	24.2 ± 9.6	
3	5	"	P_4	28.0 ± 8.3	
4	6	ov	P_4	19.2 ± 7.0	
5	4	intact	P_5	23.5 ± 3.3	
6	6	ov	P_5	20.5 ± 4.4	
7	9	ov	P_7	13.0 ± 3.8	
8	3	ov	P_8	26.0 ± 5.3	
9	12	intact	P_9		24.5 ± 2.0
10	17	ov	P_9	13.0 ± 2.4	
11	8	intact	P_{10-13}		22.8 ± 3.5
12	7	ov	P_{11-15}		17.3 ± 3.0†
13	4	ov	P_{13}	21.5 ± 4.4	
14	5	ov	P_{19}		18.6 ± 4.7†

* P_1—Day sperm in vagina. ov = ovariectomized.
† 1 μg estrone given 2 to 4 days before autopsy.

TABLE III. Overall Reproductive Performance of PMS-HCG-Treated Immature Rats.

		No. intact rats		No. ovariectomized rats		
Exp No.	No. mated/ No. treated	Mated	With sites or blast.	Mated	With sites or blast.	With spontaneous implantation
1	14/17	8	7	6	6	—
2	13/18	7	6	6	6	—
3	18/19	12	10	6	5	0
4	22/22	3	3	19	13	2
5	13/13	3	3	10	10	0
6	10/11	6	5	4	3	0
7	22/22	5	5	15	12	1
8	16/16	3	3	12	10	1
9	9/12	1	1	8	7	0
10	12/12	—	—	—	—	—
Total	149/162 (92%)*	48	43 (90%)†	86	72 (84%)†	4 (5.5%)‡

* % of rats mated.
† % of mated rats with blastocysts or implantation sites.
‡ % of spontaneous implantation in ov rats.

13 on P_{13} (21.5) were evidently indicative of the possibility of obtaining more blastocysts than the average of 13 in Group 10. Even the latter figure was 50% higher than that for mature animals in our laboratory. The average numbers of implantation sites in Groups 12 and 14 were twice as many as those in mature rats.

Ten IU of PMS alone, instead of 20 IU PMS plus 1.2 IU HCG, produced a mean of 9.3 ± 0.5 ova in 16 rats and the number of induced implantation sites in 10 ovariectomized rats on P_{11} and P_{14} was 7.7 ± 1.6. Thus delayed implantation can be induced in the 10 IU PMS-treated immature rats as well.

The overall reproductive performance of these immature rats is shown in Table III. Ninety-two percent of them mated. Ninety percent of the intact rats had ova, blastocysts or sites. Eighty-four percent of ovariectomized rats given progesterone with or without estrogen had blastocysts or sites. Eighty-two percent of the rats treated with PMS and HCG had cleaved ova, blastocysts or implantation sites. This result was comparable to that obtained by Strauss(5) who reported an 80% implantation rate in the rats of the same

age treated with 8 IU PMS. The incidence of mating and the number of implantation sites reported here and those recorded by Strauss are greater than those observed by earlier workers (Cole, PMS(2); Evans and Simpson, FSH or FSH plus HCG (8); Austin, PMS plus HCG(9)).

Summary. A single injection of 20 IU PMS on Day 30 and 1.2 IU HCG intravenously on Day 32 caused superovulation in the rats and, if mated on the evening of Day 32, an average of 22.8 implantation sites on Days 10 to 13 of pregnancy. Thirteen to 26 blastocysts were recovered from the uteri of the rats ovariectomized on Day 3 of pregnancy and given 2 mg of progesterone daily starting on Day 3. Implantation of these blastocysts was induced by a single injection of 1 μg estrone. An average of 17.3 sites was obtained when 20 IU PMS plus 1.2 IU HCG was used, and 7.7 sites when 10 IU PMS was given. The method for inducing superovulation provides the means for obtaining large numbers of blastocysts on Day 4 or 5 post-insemination, and large numbers of delayed blastocysts by a combination of treatments which cause superovulation and delayed implantation.

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Enhancement of Alimentary Hyperglyceridemia by Fructose and Glycerol in Man.* (31411)

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It is well established that administration of glucose either by oral or parenteral route diminishes the alimentary hyperglyceridemia (1,2,3). A similar effect is obtained with insulin(1). There is much evidence to suggest that this phenomenon is attributable to an accelerated rate of elimination of triglycerides from the circulation rather than to a decreased inflow of fat from the gut. Thus, the clearing of intravenously administered fat emulsion is impaired in experimental diabetes and can be normalized with insulin treatment (4,5). The activity of adipose tissue lipoprotein lipase is augmented by glucose(6,7) and diminished in diabetes(8). Also the fasting plasma triglyceride level is rapidly decreased on giving glucose(9,10) or insulin (10,11). On the other hand, it has been shown that glucose promotes the esterification of fatty acids in the intestine(12,13) and could thus be expected to increase the input of triglycerides from the thoracic duct into plasma.

The present study was undertaken because there was good reason to expect that the effects of fructose and glycerol on the alimentary hyperglyceridemia might be different from those of glucose. Both substances provide α-glycerophosphate for esterification of fatty acids in the intestinal mucosa, and, secondly, they stimulate the insulin secretion much less than glucose. Furthermore, it has been recently demonstrated that both fructose and glycerol are able to induce hyperglyceridemia in the rat(14,15).

Material and methods. The experiments

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