Because the red cells of some of the donor dogs were hemolyzed directly *in vitro* without evidence of agglutination, it was desirable to determine if there was fixation of complement in this type of hemolysis. Considerable difficulty was experienced in the use of an isohemolytic system. This has also been the experience of others working with isohemolysins.[2] Inactivation of the complement in these sera by heating at 55°C. for 30 minutes caused a reduction of hemolysis of from one-half to two-thirds of that seen in the active controls.

Conclusions. Isohemolytic and isoagglutinative phenomena noted in occasional dogs during plasmapheresis, appear to be related to antigenic formation of immune bodies by the recipient due to certain factors in the donors' red cells. These hemolytic and agglutinative properties persist in the serum for long periods of time and may be intensified by renewed injection of red cells. Isohemolysis of the red cells of dogs appears to depend at least in part upon fixation of complement.

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A Purified Diet Satisfactory for Growth, Reproduction and Lactation in Rats.

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The majority of investigative work on the adequacy or inadequacy of a given dietary regimen has involved principally 2 criteria: the rate of growth and the maximum adult weight attained. Osborne and Mendel[1] showed, on this basis, the improvement that had been accomplished in stock diets when growth records were compared with Donaldson's standards for the albino rat.[2] More recently, Bills' modification of the Steenbock diet* has been widely employed, and we have recently compared reproductive behavior

1 Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1926, **69**, 661.

2 Donaldson, H. H., *Wistar Institute of Anatomy and Biology*, Philadelphia, 1924, p. 275.

* Composition: Yellow maize, 57; whole milk powder, 25; linseed oil meal, 12; crude casein, 3.7; alfalfa leaf meal, 1.5; "iodized" table salt, 0.4; calcium carbonate, 0.4 %.

on this stock diet with that obtained with a purified diet containing various amounts of calcium and phosphorus.[3]

Some attempt has been made to devise diets of purified foodstuffs that will compare favorably, *over several generations,* with stock diets.[4] The results were not eminently successful.

In the work reported elsewhere[3] we fed a purified diet during the whole reproductive life of rat mothers. In order to determine the usefulness of this purified diet, we have fed it as the sole food for a period of more than 2 years, to 3 generations of rats.

EXPERIMENTAL.

The composition of the diet is:

Casein, washed repeatedly with dil. acetic acid	20.0
Dextrin, from corn starch by heating with citric acid	55.1
Lard, Swift's Silverleaf	9.0
Aqueous extract brewer's yeast, purified from protein, and dried (equiv. in B_1 to 24%, in B_2 to 8% yeast)	4.0
Wheat Germ Oil	1.6
Carotene Solution 3:1000, in corn oil	0.3
Calcium Acetate	1.0
Salts†	3.1
Rice Cellulose	5.9

The components of the dry diet were mixed not less often than every 2 weeks, and stored at 50°F. The diet was supplied, (as was also distilled water) *ad libitum.*

Five young stock females (200 gm.) were allowed to raise 3 consecutive litters (each reduced to six pups at birth) to 24 days of age. Weights were recorded (and are reported) at 21 days of age, for comparison with our earlier work. The first 2 litters were raised to 100 days of age, and mated to males similarly raised. The 2 groups of 3 consecutive litters which resulted were treated as was the first generation;—their young constituting the third generation.

In each instance the third litter was killed, and the carcasses ashed at 550°C. in an electric muffle.

The reproductive records of one group of stock controls, and of 3 generations of animals on our purified diet are given in Table I. It should be noted that in order to obtain an average growth curve for stock females we made 653 weighings of rats of varying ages,

[3] Cox, W. M., Jr., and Imboden, M., *J. Nutrition,* 1936, **11**, 147.

[4] Hartwell, G. A., *Biochem. J.*, 1926, **20**, 1273; 1927, **21**, 1076.

† Composition: NaCl, 24.50; $MgSO_4$, 9.14; $KHCO_3$, 59.20; KCl, 3.84; Fe citrate, 2.94; $CuSO_4$, 0.32; $MnSO_4$, 0.04; KI, 0.02%.

TABLE I.

Reproduction Records of Stock Controls, and Experimental Animals Receiving a Diet of Purified Foodstuffs.

Diet	Gestation No.	No. Females	Wt. at First Mating	No. Young Born	No. Available to Raise	% Raised	Av. Wt. 21 days gm.	% Ash	Wt. Gain of Mothers in cycle gm.	Total Gain over Initial Wt.	Wt. of offspring at 100 days of age ♂	Wt. of offspring at 100 days of age ♀
Stock			96.9*									
	1	10	192.2	98	60	100	39.2		41.0			
	2	10		93	57	96.4	44.5		40.7			193.0†
	3	10		107	60	86.6	45.5	2.70	22.9	103.3		
Av.				9.9	5.9	94.4	42.9		34.9			
Purified			98.2*									
Diet	1	5	205.0	55	31	100	37.6		37.2			
1st Gen-	2	5		46	27	100	44.1		30.0		280.5	200.0
eration	3	5		49	30	100	46.7	2.74	33.5	94.6		
Av.				10.0	5.9	100	42.7		33.6			
Purified			100.8*									
Diet	1	9	202.7	94	54	100	41.3		42.8			
2nd Gen-	2	9		82	53	94.3	42.8		29.0		291.6	195.9
eration	3	9		91	54	85.2	46.3	2.61	25.4	77.3		
Av.				9.9	6.0	93.2	43.2		32.3			
Purified			99.8*									
Diet	1	19	195.9	140	90	78.9	41.8		34.0			
3rd Gen-	2	19		161	100	89.0	42.5		25.2		290.5	194.0
eration	3	19		150	92	90.2	44.2	2.68	26.3	83.2		
Av.				8.8	5.5	86.2	42.9		28.6			

*Age in days at first mating.

†Computed from growth records of 66 stock female rats.

—all housed in our stock rattery, and saved for breeding purposes. Only the larger rats (at 24 days of age) are saved for breeding.

From Table I it is evident that reproduction and lactation on a purified diet can be the equal of that on a mixed stock diet. Commonly, 3 criteria may be employed to determine the adequacy of diets:—the weight of nurslings at 21 days of age, the gain in weight of the mother, and the ash content of the young.[3] For the stock controls and experimental animals, first, second, and third generations, respectively, we have: weight of young at 21 days,—42.9, 42.7, 43.2 and 42.9 gm.; final adult weight of the mothers, —295.5, 299.6, 280.0 and 279.1 gm.; gross body ash,—2.70, 2.74, 2.61 and 2.68%. The differences are so small that we can attribute no significance to them.

From the standpoint of reproduction there appears to be a small decrease in the number of young born in the third generation: the controls averaged 9.9 pups per mother per litter, while the third generation averaged but 8.8 per litter. Many factors determine the number of young born, but, in the present experiment, as all young born were living, and of average weight at birth, we cannot attribute much importance to the observed difference.

The percentage of young raised showed a small decrease in the third generation, as compared with the controls—in spite of the fact that the average weight of nurslings at 21 days was the same. We are inclined to discount this small difference in view of the fact that most of the young in this generation were born in the months May to September, whereas the 3 control litters were born October to February.

Table I gives the weights of the 3 generations of males and females on the experimental diet, at 100 days of age as compared with the average weight of stock females. It is evident that there is no difference between adult weights of these 3 generations of experimental rats, when compared with the weight of stock females.

Summary. Three generations of rats have been raised to maturity while receiving a diet composed solely of purified foodstuffs. It is shown that growth, reproduction and lactation on this diet are as satisfactory in every way as when a standard stock diet is employed.