ciency of biotin, pantothenate, or *l*-tryptophane.

Though the total fermentation of carbohydrate is reduced when biotin, pantothenate, or nicotinic acid are present in suboptimal amounts, the fact that the end product is always lactic acid suggests that the main pathways of carbohydrate metabolism are unchanged regardless of the growth factor to which the organism is responding.

Summary. 1. The turbidity produced by the growth of *Lactobacillus arabinosus* is greater in relation to acid production when the organisms are responding to suboptimal amounts of nicotinic acid than when they are responding to suboptimal amounts of biotin, pantothenic acid, or *l*-tryptophane.

2. All the acid produced by the *Lactobacillus arabinosus* is lactic acid regardless of whether the bacteria are responding to nicotinic acid, biotin, or pantothenate.

15173

Changes in Erythrocytes of Hamsters Following Castration, Splenectomy, and Subsequent Liver, Iron, and Testosterone Injections.

KATHRYN F. STEIN AND EDITH CARRIER. (Introduced by A. Elizabeth Adams.)

From the Zoology Department, Mount Holyoke College.

A variety of evidence indicates that the male sex hormone has a stimulating effect upon the red blood cell count in mammals. It is well known that men have a higher average count than women, and a similar sex difference has been found to occur in many other mammals including rats,[1] rabbits,[2] mice,[3] and cats.[4] That this is due, in part at least, to the effect of the male hormone has been demonstrated by the increased count following testosterone injections in hypogonadic men,[5] in capons,[6] and in castrate female and hypophysectomized rats,[7] and by the reduction in number of red blood cells following castration in the hamster (*Cricetus auratus*) reported previously from this laboratory.[8] Some progress has also been made toward an understanding of the mechanism by which the effect of the male hormone is produced. Vollmer and Gordon[7] found increased reticulocytosis and hyperplasia of the bone marrow in hypophysectomized rats injected with testosterone propionate. The present study was undertaken to confirm and extend the results obtained following castration in the hamster[8] and also to attempt to discover further details concerning the processes involved in the effects of castration upon the red blood cells.

Experimental. Nineteen male golden hamsters (*Cricetus auratus*) (Tables I, II) were employed in this experiment, four sets of littermates plus 2 animals from the same strain but of which the exact relationship to each other was unknown. All were of the fourth and fifth generations from an original inbred litter of four animals born in this laboratory in the spring of 1943. They were kept in a temperature of 70 to 75°F and had Purina Dog Chow and/or Pratt's Nurishmix constantly available in their cages, supplemented occasionally by milk and lettuce. They appeared

1 Adams, A. Elizabeth, and Shevket, Fazilé, *Physiol. Zool.*, 1929, **2**, 181.

2 Rosahn, Paul D., Pearce, Louise, and Hu, Ch'uan K'uei, *J. Exp. Med.*, 1934, **60**, 687.

3 Kamenoff, Ralph J., PROC. SOC. EXP. BIOL. AND MED., 1936, **36**, 411.

4 Lewis, Lena A., *Endocrinol.*, 1941, **28**, 821.

5 McCullagh, E. P., and Jones, R., *Cleveland Clin. Quart.*, 1941, **8**, 79.

6 Taber, Elsie, Davis, David E., and Domm, L. V., *Am. J. Physiol.*, 1943, **138**, 479.

7 Vollmer, Erwin P., and Gordon, Albert S., *Endocrinol.*, 1941, **29**, 828.

8 Stein, Kathryn, and Jacobsen, Barbara, *Anat. Rec.*, 1944, **88**, 459.

TABLE I.
Changes with Age in the Red Blood Cell Values of Hamsters.

Age in days	RBC count: Animal No.	RBC count: No. of animals	RBC count: Avg No. RBC and range in millions	RBC vol.: No. of animals	RBC vol.: Total vol. in %	RBC vol.: MCV in μ^3	Hemoglobin: No. of animals	Hemoglobin: Total Hb. g 100 cc	Hemoglobin: MCHb in $\gamma\gamma$
20	1, 2	2	5.06 (5.03-5.08)	—	—	—	—	—	—
33-34	3-7	5	5.96 (5.36-6.69)	1	47.5	70.8	—	—	—
36-37	8, 12, 13, 14	4	6.36 (5.87-6.55)	—	—	—	3	17.04	27.1
40-43	3, 7, 14	3	6.98 (6.40-7.55)	2	45.3	67.5	1	17.8	23.6
47-50	3, 7, 14	3	7.94 (7.84-8.01)	2	43.8	55.2	1	18.3	22.9
57-62	3, 7, 14-17	6	8.60 (8.16-9.29)	6	52.9	63.2	4	17.38	20.9
66-70	3, 7, 9-11	5	8.88 (8.32-9.03)	4	48.6	55.1	4	18.94	20.4
85-100	3, 7, 11, 14, 15	5	9.14 (9.06-9.02)	5	52.2	57.1	4	18.26	19.9
108-131	3, 7, 11, 14, 15	5	9.17 (9.14-9.20)	5	53.2	58.0	2	15.65	17.1

to be in healthy condition throughout the experiments.

Castration was performed on 8 animals; 1 was operated at 20 days of age, 4 at 33 to 37 days, and 3 at 66 to 73 days (Table II). Controls were subjected to similar operative procedure up to the point of actual removal of the testes. Later in the experiment 4 of the castrates, all adult and with approximately the same number of red blood cells, and 5 controls, also adult, were splenectomized (Table II). Since no significant change had occurred due to operative procedure in the controls for the castrate animals, and since the removal of the spleen was accomplished with no greater loss of blood from hemorrhage, controls for the latter series were not operated. For all the operations 0.1 cc of a 10% alcoholic solution of Nembutal (Abbott), containing 1 gram per cc, was injected intraperitoneally as a preliminary anesthetic and followed by ether as needed.

Attempts to restore the counts of operated animals to normal were made by injections of liver extract or of iron citrate. The injected animals in each of the 2 groups, liver and iron, included 2 castrates, 1 splenectomized "control," 1 splenectomized castrate, and the same normal control (Table III). The liver (from Lederle Laboratories and containing 15 U.S.P. injectable units per cc) was administered in daily intramuscular injections of 0.1 cc or 1½ units to each animal for 3 days. Counts were taken within 12 hours after the last injection. Eli Lilly iron citrate, containing 0.05 grams per cc, was injected in 0.1 cc quantities into each animal of the "iron" group daily for 3 days and counts taken within 6 hours after the last injection. Finally a series of 9 hamsters, 2 castrates, 3 splenectomized castrates, and 4 splenectomized "controls," were injected with 0.1 cc of a preparation containing one mg of propionic ester of testosterone per cc (Oreton Schering), daily for 3 days and counts taken the day following that of the last injection (Table IV).

Blood for all determinations was secured from the tails of animals under light ether anesthesia. An interval of a week was usually allowed between determinations to avoid the possibility of anemia from excess bleeding. A modified Hayem's solution recommended for rats by Smith[9] was used as the diluent. Counts were made of 4 chambers and averaged for each reported count prior to the splenectomy operations; since the coefficient of variation was usually under 1% only 2 chambers were counted thereafter. Volume determinations were made in a Van Allen capillary hematocrit centrifuged at 2600 r.p.m., and hemoglobins were read in a colorimeter equipped with a Newcomer disc.

Results. Normal Levels of Young and Adult Hamsters. The number of red blood cells in the hamster increases with age (Table I). The data confirm the results previously reported on a smaller number of animals.[8] The adult value of approximately 9 million per cc was a strikingly uniform result in all animals counted, and since it was also maintained in weekly counts up to the age of

[9] Smith, Christianna, *J. Path. and Bact.*, 1932, **35**, 717.

TABLE II.
Effects of Castration and Splenectomy on the Erythrocyte Count.

Animal No.	Age at operation		Number of RBC in millions		
	Castration	Splenectomy	Before op.	After castration	After splenectomy
1	20 days	119 days	5.08	6.41	6.52
4	33	—	5.37	6.54	—
8	37	—	6.52	6.57	—
12	36	114	6.55	6.58	6.54
13	36	114	5.87	6.57	6.54
16	66	114	8.85	6.55	6.55
17	66	—	8.64	6.54	—
9	73	—	9.00	6.56	—
			Avg	6.55	6.54
3	—	119	9.20	—	5.73
11	—	137	9.19	—	6.52
14	—	114	9.15	—	6.54
18	—	mature	—	—	6.52
19	—	”	—	—	6.56
				Avg	6.37

165 days (Stein and Jacobsen, 226 days) it is considered as the normal level for adult hamsters of this species. Increases in total red blood cell volume also occurred with age but not proportional to the increase in number of cells; consequently the size of the individual cells was perceptibly decreased. Variations in total hemoglobin were neither great nor consistent and mean corpuscular values were higher in young animals (Table I).

Effects of Castration and Splenectomy. A level of approximately 6.5 million cells per cu mm, as compared with the normal count of 9 million, was eventually attained by all castrated animals regardless of the age at which the operation was performed (Table II). In adult animals this level was attained within a two-week period following castration and values within a range of 6.41 to 6.59 million were maintained for the 8-week period of counting. Counts for animals castrated before maturity rose to the 6.5 level and were then maintained for 1 to 6 weeks, that is, until other treatments were initiated or counting discontinued.

Removal of the spleen was followed by a marked reaction in normal control animals as compared with the lack of effect on castrates. The count for the former had dropped to an average of 6.4 million 2 weeks after the operation and this level was maintained for 6 weeks by the one animal for which weekly counts were made over so long a period. Also a

TABLE III.
Effects of Liver and Iron Injections on Red Blood Cells of Hamsters.

Animal No.	Operation	Injection	No. of RBC in millions		Size of RBC in μ^3	
			After op.	After inj.	After op.	After inj.
17	Castration	liver	6.54	9.06	—	—
8	”	”	6.55	9.14	80.9	54.7
11	Splenectomy	”	6.52	9.13	76.7	58.1
16	Castra. + splen.	”	6.54	9.19	76.3	53.3
15	Control	”	9.14	9.15	54.7	60.1
4	Castration	iron	6.54	8.85	85.6	—
9	”	”	6.56	9.11	81	—
18	Splenectomy	”	6.52	9.17	75.2	54.5
13	Castra. + splenec.	”	6.57	9.07	84.5	59.3
15	Control	”	9.14	9.21	54.7	57.5

TABLE IV.
Effects of Testosterone Injections on Red Blood Cells of Hamsters.

Animal No.	Operation	No. of RBC in millions Before testosterone	No. of RBC in millions After testosterone	Size of RBC in μ^3 Before testosterone	Size of RBC in μ^3 After testosterone
8	Castration	7.52	9.18	—	49.0
17	"	7.73	9.12	—	—
3	Splenectomy	8.09	7.60	64.1	—
11	"	7.63	7.62	—	61.7
14	"	7.96	7.56	61.7	64.5
19	"	6.54	6.52	—	65.9
1	Castr. + splen.	8.25	7.34	63.0	62.7
12	"	7.01	7.62	65.6	64.3
16	"	7.64	7.66	—	63.9

count of 6.49 was made on the blood of another animal dying 10 months after splenectomy.

The average size of the erythrocytes increased both after castration and after splenectomy, in the former case to 81.2 (74.5-84.1) cubic micra, in the latter to 75.4 (70.3-80.3). Normal controls had an average cell size of 56.5 (51.4-60.9) cubic micra. Splenectomy of castrates was followed by a slight decrease, to 76 cubic micra, but it is doubtful if this difference is significant. In general the effects of splenectomy were essentially the same as those of castration.

Total hemoglobin values averaged slightly higher in castrate than in control animals but the difference was too slight to be significant. The amount of hemoglobin per individual cell, however, was definitely increased, ranging from 27 to 31 micromicrograms in castrates and from 17 to 21 in controls. The effect of splenectomy upon the total amount of hemoglobin in the blood of the hamster cannot be determined at this time. Data on individual animals, "normal" and castrate, before and after splenectomy, indicate a definite drop in amount of hemoglobin per 100 cc of blood both at one and two weeks after operation. However, unoperated controls, normal and castrate, showed a similar decrease when determinations were made on the same dates as those for the splenectomized animals. This may perhaps indicate a seasonal variation in hemoglobin.

Effects of Injections. Liver or iron citrate injected into the hamsters with lower than normal red blood cell counts resulted in an immediate rise of the number to normal, whether the animals had previously been castrated, splenectomized, or both (Table III). These increases were noted within 6 to 12 hours after the last of 3 daily injections of the liver or iron. Along with the increased count a decrease occurred in the size of the individual cells (Table III).

Testosterone injected for a similar period, 3 daily injections, produced changes only in the castrated hamsters. None of the splenectomized animals, whether or not they had been castrated, showed any response in counts made the day following the last injection. The animals which received these injections had been treated previously with other substances and there had not been enough time for their counts to return to the level characteristic for their condition when the testosterone was injected. However, the effect, or lack of effect, of testosterone was obvious and consistent (Table IV).

In most of the splenectomized animals the number of red blood cells continued to drop toward the splenectomy level in spite of the testosterone injections and in none of them was there an increase in the least approaching the spectacular rise manifested by both of the castrates. The individual red blood cells remained macrocytic in all the splenectomized animals, castrated and uncastrated, injected with testosterone. In castrated "controls," however, the injections were followed by a decrease in average cell size in the one animal of this type (No. 8, Table IV) for which a volume determination was made.

Discussion. The results obtained on hamsters in this study agree with those of other workers in the field upon other animals in

demonstrating a definite effect of the male sex hormone upon the number of red blood cells present in the circulating blood. As reported previously[8] castration caused approximately a 25% decrease, from 9 to 6.5 million per cu mm, in the number of cells. This is similar to the decrease from 3.6 to 2.51 million observed by Taber, Davis, and Domm[6] in capons. Injections of testosterone raised the number in castrated hamsters, as it did in capons[6] and in hypophysectomized rats.[7,10] In the latter the effect of the male hormone was noted as due to increased erythropoiesis. Because of the small amount of material available this has not been confirmed for the hamster.

Young animals responded somewhat differently from adults in that such animals, if they were castrated before the number of red blood cells had reached the adult castration level, showed an increase to that level. The final result was the same, a castration level of approximately 6.5 million cells per cu mm. The regulation of the gradual increase of the red cell count in young animals up to this level is apparently controlled by other than sex factors.

It is interesting that the change in the number of cells due to the influence of sex hormone was accompanied by a change in the size of the individual cells so that the total volume of red blood cells in the circulating blood was essentially the same in castrated as in normal animals. As far as our investigations go they seem to indicate that the total amount of hemoglobin was also kept at a relatively normal level. The increase in the amount of hemoglobin per cell in the castrates compensated on the whole for the decreased number of cells. The regulatory effect on the cell size and content thus seemed to be, in the hamster, the main effect of the male sex hormone.

The influence of the spleen on the blood picture has been the subject of numerous investigations. Both splenectomy and injections of splenic extract have indicated that the spleen may exert a stimulating effect upon the formation of red blood cells.[11,12,13] Splenectomy of hamsters in the present study produced the same decrease in the total number and the same increase in the size of individual cells as was observed following castration. This similarity and the fact that splenectomy of castrated animals caused no further changes in the red blood cells suggested a possible interrelationship between the testis and the spleen. Evidence for such a relationship was furnished by the experiments in which injections of male hormone were made. No effect of testosterone on the number or size of red blood cells was observed in splenectomized animals, whether or not they had been castrated. The effect of male hormone in changing the count was apparently not exerted directly upon the bone marrow but upon the spleen. It is possible perhaps that the increase in number following testosterone injections was due, not to an indirect effect through the spleen on erythropoiesis, but merely to a direct stimulation of the spleen to release hoarded cells. Injections over longer periods than the 3 days of the present study might give different results. However, the positive results obtained with the use of liver or iron extract after a similar short period of injection at least indicate that the bone marrow is capable of response within that period.

It is further indicated from the results of injections of liver and iron that the effect of the spleen on the number and size of the red blood cells is tied up with the liver and/or iron supply since either liver extract or iron citrate was effective, within a period of 3 to 4 days, in restoring a normal count and a normal cell size in all operated animals, castrated, splenectomized, and those which were both splenectomized and castrated. It is difficult to understand why both substances should have the same effect. Liver deficiency anemias give a macrocytic picture as compared with the small size of the cells which ordinarily occur following iron deficiency.[14] As noted previously, however, injections of iron citrate

[10] Vollmer, Erwin P., Gordon, Albert S., and Charipper, Harry A., *Endocrinol.*, 1942, **31**, 619.

[11] Krumbhaar, E. B., and Musser, J. H., *J. Exp. Med.*, 1914, **20**, 108.

[12] Krumbhaar, E. B., and Musser, J. H., *Arch. Int. Med.*, 1923, **31**, 686.

[13] Eddy, Nathan B., *Endocrinol.*, 1921, **5**, 461.

[14] Minot, George R., *J. Am. Med. Assn.*, 1935, **105**, 1176.

as well as those of liver caused return of cell size and number to normal in the operated hamsters. It is true, of course, that the picture produced in the hamster following either or both operations may not be that of a true anemia, since the total volume of cells remains practically normal. Whether this is also true of the total hemoglobin cannot yet be stated definitely due to the complication of the results either by a seasonal variation of the pigment or by variation of technic. Krumbhaar[15] however makes no distinction in kind, but points out that there are variations in degree and in time of appearance and duration of anemia following splenectomy of different animals. He suggests that the anemia is due to a loss with the spleen of a property which aids erythrocyte formation, such as the conservation of the iron of broken down erythrocytes. And Balliere[16] noted that following splenectomy in the dog the iron content of the plasma dropped with a corresponding decrease in number of red blood cells. Presumably then the iron citrate injections may substitute for the absence of the spleen and may directly affect bone marrow. It is questionable whether the similar action of liver extract in this experiment can be attributed to its iron content since it is known that liver extract is not effective in remedying human anemia due to iron deficiency. However, perhaps the difference is a quantitative one. The only alternative explanation, if the same mechanism is supposed to be set in motion by the two types of injections, is that the injected iron has an effect on the liver of the hamster resulting in the liberation of some liver substance more directly responsible for the effects on the bone marrow. Krumbhaar[17] has also suggested that the spleen may have the mission of activating liver function.

Gordon *et al.*[18] questioned whether a function of the spleen might be to regulate the structure of the red cells as they are produced from the marrow. Certainly this study seems to indicate an influence of the spleen, and perhaps indirectly of the male hormone, on the regulation of the volume of the individual red blood cell.

Summary. Castration of hamsters caused a decrease in the number (25-30%) and in the size of erythrocytes. In animals castrated when young enough to have fewer cells than the number typical for castrates the count increased up to the castrate level. Splenectomy had the same effects as castration upon the number and size of red blood cells and no additional change occurred when castrated animals were splenectomized. Liver extract or iron citrate injected daily for three days restored a normal count and normal average corpuscular volume in all operated animals, castrates, splenectomized, and splenectomized castrates. Testosterone injected for a similar length of time was effective in castrates only and was ineffective in all splenectomized animals.

[15] Krumbhaar, E. B., *Am. J. Med. Sci.*, 1932, **184**, 215.

[16] Balliere, A., *Biol. Abs.*, 1941, **152**, 1640.

[17] Krumbhaar, E. B., *Physiol. Rev.*, 1926, **6**, 160.

[18] Gordon, Albert S., Kleinberg, William, and Ponder, Eric, *Am. J. Physiol.*, 1937, **120**, 150.