volved in the isolation, this result means that a minimum of 16% of the administered dose was lost in the urine in the first 24 hours. This extreme clinical inefficiency might be overcome by a preliminary period of phosphorus starvation since it has been observed in this laboratory that rats on a high calcium-low phosphorus diet reduce their urinary output of radioactive phosphorus to about 1/60th of its normal value. If such a diet were employed preliminary to the clinical use of radiophosphorus it would be necessary to administer the active phosphorus by a parenteral route or, if the oral route is used, to subject the patient to a preliminary fast sufficiently long to clear the intestine of calcium.

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Experimental Production of Target Cells by Splenectomy and Interference with Splenic Circulation.*

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(Introduced by J. H. Pratt.)

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Target cells are erythrocytes which in the stained blood film show a central mass of hemoglobin within an unstained intermediate zone which is in turn surrounded by a peripheral rim of hemoglobin, thus giving the appearance of a "bull's eye" or target. Haden and Evans[1] first described these cells as "Mexican hat" cells and stated they were characteristic of sickle cell anemia. Barrett,[2] who suggested the term "target cell", found them in increased numbers in obstructive jaundice, certain cases of hypochromic anemia, in steatorrhea, and following splenectomy. He also demonstrated that target cells were abnormally thin and showed an increased hypotonic resistance. Recently these cells were described by Dameshek[3] and by Wintrobe, *et al.*,[4] as the outstanding hematological feature of a

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1 Haden, R. L., and Evans, F. D., *Arch. Int. Med.*, 1937, **60**, 133.

2 Barrett, A. M., *J. Path. and Bact.*, 1938, **46**, 603.

3 Dameshek, W., *Am. J. Med. Sci.*, 1940, **200**, 445.

4 Wintrobe, M. M., Matthews, E., Pollack, R., and Dobyns, B. M., *J. A. M. A.*, 1940, **114**, 1530.

hitherto undescribed type of hereditary anemia probably related to Cooley's anemia and found in adult Italians.

The occurrence of target cells following splenectomy, and in sickle cell anemia in which the spleen not infrequently becomes atrophic, points to a possible relationship of these cells to splenic function. In a previous study,[5] target cells were almost regularly demonstrated (unless spherocytosis persisted) in 19 patients splenectomized for various conditions. It was evident that these cells might remain in considerable numbers for several years after removal of the spleen. In the present study, an attempt has been made to produce target cells experimentally either by splenectomy or by interference with the splenic blood supply.

Methods. Splenectomy was performed in dogs, guinea pigs and rabbits. Interference with the splenic circulation was performed in dogs and consisted either of almost complete elimination of the arterial blood supply, or of complete ligation of all the splenic veins with the exception of some anastomoses with the gastric vessels. In a few experiments incomplete ligation of the splenic veins was performed. Determinations of the hemoglobin, red cell count, white cell count, platelet count, hypotonic (sodium chloride) fragility, and of the percentages of target cells and Howell-Jolly bodies (among 1000 erythrocytes) were made. In the animals studied, target cells were either lacking or present in small numbers (less than 1%); Howell-Jolly bodies were not normally present.

Results. Dogs. Large numbers of target cells were regularly observed after splenectomy in 3 dogs. In "Yellow Dog," which was studied for 162 days after splenectomy, the number of target cells remained below 20% during the first 2 months but increased later (109 to 134 days) to levels between 40 and 70%. There was an associated change in the "minimum" value of the hypotonic fragility, from .26 to .12%, indicating an increased resistance of certain cells, later followed by a change in the "maximal" fragility, from .56 to .46%. Howell-Jolly bodies increased concomitantly with the target cells.

Complete ligation of the splenic veins in 2 dogs likewise led to a considerable increase of target cells, which was accompanied by a slight increase in the hypotonic resistance. Howell-Jolly bodies were, however, lacking or found only in very small numbers.

Incomplete obstruction of the splenic veins was also followed by the development of target cells but in considerably smaller numbers than following the other operations. Ligation of the splenic artery in

[5] Singer, K., Miller, E. B., and Dameshek, W., *Am. J. Med. Sci.*, 1941, **202**, 171.

2 dogs was followed by a slight inconstant increase of target cells. Howell-Jolly bodies were lacking.

Guinea Pigs. Splenectomy in 6 guinea pigs was followed by the development of target cells but in considerably smaller numbers than in dogs, the highest value encountered being little more than 10%. There was also a concomitant decrease of the minimum saline fragility and the appearance of Howell-Jolly bodies.

Rabbits. Splenectomy in 3 rabbits failed to result in target cells or in a change of the hypotonic fragility during periods of observations up to 120 days. Howell-Jolly bodies were, however, observed.

Discussion. The appearance of considerable numbers of target cells following both splenectomy and complete splenic vein ligation point either to a direct influence of the spleen upon the erythrocytes passing through it or to a hormonal effect upon the production of red cells in the bone-marrow. It is conceivable that the red cells upon their production in the bone-marrow are relatively thin (target cells) and develop a "normal" degree of thickness in the process of passage through the sinusoids of the spleen. Numerous studies[6] bearing directly or indirectly on the problem, indicate that the red blood cells

TABLE I.
"Yellow" Dog.
Ligation of Splenic Artery; Splenectomy.

Date	Days preligation	R.B.C. (millions)	Hgb. %	W.B.C.	Target cells %	Howell-Jolly bodies %	NaCl fragility %
	33	4.0	69	19,000	0	0	.56-.26
	31	3.9	64	19,200	0	0	—
	16	4.2	71	19,000	0	0	.48-.28
			Ligation of Splenic Artery.				
	Postligation						
11/8/39	2	4.1	63	24,600	0	0	.44-.24
11/16	10	4.6	73	21,300	1.0	0	.46-.28
11/23	17	3.8	61	29,000	4.4	0	.52-.22
			Splenectomy.				
	Postsplenectomy						
12/1	0	4.3	67	48,200	2.6	0	.50-.34
12/22	21	4.9	72	25,500	6.3	.3	.44-.16
1/4/40	33	4.3	75	—	8.0	.4	.50-.20
1/16	45	4.7	76	19,900	18.0	.6	.42-.20
1/30	59	4.0	71	19,200	16.7	.4	.50-.12
3/20	109	4.8	84	12,300	72	.7	.44-.16
3/29	118	4.4	82	—	40.8	1.2	.46-.20
4/15	134	5.3	86	—	50.6	.9	.46-.26
4/24	143	5.0	75	18,400	31.4	.5	.42-.22
5/13	162	4.6	81	20,300	36.2	.8	.46-.24

[6] Ham, T. H., and Castle, W. B., *Proc. Am. Philos. Soc.*, 1940, **82**, 411; Knisely, M. H., (a) *Anat. Rec.*, 1936, **65**, 23; (b) *Ibid.*, p. 131; Lauda, E., *Die normale und pathologische Physiologie der Milz*, Urban & Schwarzenberg, 1933.

TABLE II.
Guinea Pig No. 80.
Splenectomy.

Days presplenectomy	R.B.C. (millions)	Hgb. %	W.B.C.	Platelets (×000)	Target cells %	Howell-Jolly bodies %	NaCl fragility %
23	5.6	96	13,100	1,845		0.0	.40-.24
16	5.2	80	15,100	1,967	0.2	0.0	.42-.16
			Splenectomy.				
Postsplenectomy							
7	4.3	71	10,800	2,812	1.8	1.5	.44-.16
32	5.2	90	29,200	2,021	1.8	1.5	.42-.20
34	4.6	65	29,900	1,829	7.0	0.9	—
39	4.3	73	24,500	1,450	7.7	1.9	—
						Norm 1	
41	4.3	71	23,300	2,888	8.0	1.9	—
						Norm 1	
63	5.3	78	24,900	2,421	5.3	0.7	—
70	5.0	77	18,800	1,355	10.6	0.4	.34-.04
80	5.3	80	23,100	—	8.6	0.3	.34-.12

become modified in their passage through the spleen and that the modification is in the direction of increased spheroidicity and increased hypotonic fragility. In the absence of the spleen, it is likely that the red cell population tends on the whole to be thinner than normal, a definite proportion of erythrocytes becoming sufficiently thin to be recognized as target cells. It is likewise possible that the spleen exerts some influence upon the last stages of erythrocyte maturation and that in the absence or diminution of such a hypothetical splenic maturation principle target cells may develop. Such a hormonal influence seems probable as Hirschfeld and Weinert[7] first suggested, in the explanation of the development of Howell-Jolly bodies.

Summary. Target cells (abnormally thin erythrocytes) appeared in considerable numbers following splenectomy in dogs and guinea pigs and complete ligation of the splenic veins in dogs. In rabbits, splenectomy failed to result in the development of target cells. The spleen either has a direct effect on the red cells passing through it or a hormonal effect on the last stages of erythroblastic maturation in the bone-marrow.

[7] Hirschfeld, H., and Weinert, H., *B. klin. Wschr.*, 1914, **22**, 1026.