Orally ingested histidine does influence the amount of histidine excreted but the percentage of the ingested dose appearing in the urine seems to be more dependent on the individual subject than on the amount of the amino acid ingested, *e.g.*, the subject receiving 7.5 g of histidine excreted 6.3%, while the 3 subjects receiving 10 g excreted 3% or less.

Studies of the urinary content of other imidazoles after histidine administration to human subjects are in progress.

Summary. Oral L-histidine given to human subjects fed weighed diets constant in protein content had no consistent effect on the urinary content of 1-methylhistidine and 3-methylhistidine; the amounts of carnosine excreted increased somewhat, while anserine excretion decreased. The methylated forms of histidine, either free or combined in the dipeptide anserine, do not appear to be primary urinary end-products of histidine metabolism in human beings.

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Antibody Response in Splenectomized Monkeys.* (29360)

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Previous studies from this laboratory(1) and elsewhere(2-4) showed that antibody response in splenectomized humans after subcutaneous inoculation of particulate bacterial antigens was usually not different from that in non-splenectomized controls. Rowley(5), however, observed a depressing effect of splenectomy on hemolysin production in humans inoculated intravenously with sheep erythrocytes. Similarly, intravenous inoculations of heterologous erythrocytes in splenectomized rabbits(6,7) and rats(8,9) were followed by poor antibody response. This led to the assumption that splenectomy may depress antibody response after intravenous, but not after subcutaneous inoculation of antigen(10).

This report compares antibody response of intact and splenectomized monkeys after intravenous inoculation of typhoid vaccine and sheep erythrocytes, and after subcutaneous inoculation of tularemia and typhoid vaccines.

Materials and methods. Young pre-pubertal monkeys (*Macaca mulatta*) were splenectomized under pentothal-ether anaesthesia, and search made for accessory spleens.† Antigens used were triple-washed 2% or 20% sheep erythrocytes, triple typhoid vaccine (Wyeth) diluted to contain 25 or 50 million typhoid bacilli per ml, and Foshay phenolized tularemia vaccine containing 7.5 billion organisms per ml.† Intravenous inoculations were made into the saphenous vein, and subcutaneous inoculations in the lateral thigh. Blood for serologic study was drawn from the femoral vein before inoculation, and at 12 weekly intervals. Tularemia agglutination tests were performed as previously described(11). Typhoid "H" and

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TABLE I. Antibody Response of Splenectomized and Non-splenectomized Rhesus Monkeys After Intravenous Inoculation of Sheep Erythrocytes.

Exp	Antigen, dose	Weeks post-splen.	Test*	Titer rise† Splenectomized				Not splenectomized		No. with 4-fold or more rise Splen.	Not splen.
1	2% sheep RBC 1.0 ml I.V.	2	NA	4/4	2/2	5/5	2/2	*4/6*‡	3/6	0/4	2/2
			A	0/0	0/0	0/0	0/0	0/4	0/3	0/4	2/2
			H	4/4	*2/4*	3/3	*2/3*	*4/6*	3/7	1/4	2/2
2	*Idem*	6	NA	*4/5*	4/4	5/5	4/4	1/6	1/3	0/4	2/2
			A	0/0	0/0	0/0	*0/2*	0/5	0/2	1/4	2/2
			H	*4/6*	*2/5*	6/6	5/5	2/8	5/5	2/4	2/2
3	"	14 (Boost)§	NA	*3/4*	2/2	*3/5*	4/4	4/6	4/5	1/4	1/2
			A	0/0	0/0	0/0	*0/3*	0/6	0/4	1/4	2/2
			H	*5/6*	*3/5*	*5/7*	4/4	4/6	6/6	2/4	1/2
4	20% sheep RBC 1.0 ml I.V.	14 (Boost)§	NA	5/6	2/2	5/5	4/4	4/6	0/5	0/4	2/2
			A	0/3	0/0	0/0	0/0	0/6	0/5	1/4	2/2
			H	3/7	*2/4*	6/6	4/5	4/7	0/6	2/4	2/2
5	*Idem* × 3 days	2	NA	*3/5*	1/4			3/7		2/2	1/1
			A	0/0	0/0			0/7		0/2	1/1
			H	*3/4*	*1/7*			5/9		1/2	1/1
6	" "	6	NA	*0/3*	*2/4*			0/4		2/2	1/1
			A	0/0	0/2			0/4		1/2	1/1
			H	*3/5*	*0/6*			0/6		2/2	1/1

* NA, A = Agglutination, serum not absorbed or absorbed with guinea pig kidney, respectively; H = hemolysin.

† Numerator = base line titer; denominator = peak titer of each animal. Titers of 0, 5, 10, 20, etc., to 1280 coded as 0, 1, 2, 3, etc., to 9, respectively.

‡ Fraction italicized = peak titer 2 wk post-inoculation or later; not italicized, 1 wk.

§ Same monkeys inoculated 2, 6 wk post-splenectomy.

"O" tube agglutination tests were carried out by standard methods(12), as were hemolysin titrations(13) and erythrocyte agglutination tests on serum not absorbed, and absorbed with guinea pig kidney(12), with the exception that the latter tests were centrifuged for 3 minutes at 1400 rpm in an International Size 2 centrifuge to facilitate reading.

Results. As seen in Table I (Exp. 1), after intravenous inoculation of 1.0 ml of 2% sheep erythrocytes 2 weeks post-splenectomy, none of 4 monkeys showed agglutinin titer rise while both controls exhibited 4-fold or greater increases. Pre-inoculation natural anti-sheep cell titers from 1:10 to 1:80 observed in sera of these 6 monkeys were completely removed after absorption with guinea pig kidney. As seen in Table I, post-inoculation sera from all 4 splenectomized monkeys were non-reactive after absorption, while both controls showed significant titer rise. Significant rise in hemolysin titer was observed in only 1 of 4 splenectomized and in both controls. Four additional monkeys (Table I, Exp. 2) were given the same inoculum 6 weeks post-splenectomy. None of 4 showed significant agglutinin rise with unabsorbed sera, but 1 of 4 did show a 4-fold increase at 2 weeks with absorbed sera. This monkey showed no significant hemolysin titer increase but 2 of the remaining 3 did. Both controls reacted significantly in both agglutination tests and 1 of 2 showed significant change in hemolysin titer.

The 12 monkeys from Exp. 1 and 2 were reinoculated, intravenously, 8-12 weeks later (14 weeks post-splenectomy) with 1 ml of 2% or 20% sheep erythrocytes. After 2% cell booster 1 of 2 controls showed significant titer increases in all 3 tests, while one showed significant rise in absorbed agglutination titer only (Table I, Exp. 3). In the splenectomized group significant titer rises were observed in both non-absorbed agglutination and hemolysin tests in 1 of 4 while 1 monkey each did so only in hemolysin or

TABLE II. Agglutinin Response of Splenectomized and Non-splenectomized Rhesus Monkeys to Bacterial Antigens Administered Subcutaneously or Intravenously.

Exp	Antigen, dose	Weeks post-splen.	Titer rise†						No. with 4-fold or more rise	
			Splenectomized				Not splenectomized		Splen.	Not splen.
1	Tularemia vaccine, .5 ml, S.C.	2	0/5	0/5	0/4	0/5	0/5	0/4	4/4	2/2
2		6	0/4	0/4	0/3	0/4	0/5	0/5	4/4	2/2
3	Typhoid vaccine,* .5 ml, S.C.	10	0/4	0/3	0/4	0/5	0/3	0/3	4/4	2/2
4	Typhoid vaccine, 1.0 ml, I.V.	2	0/1	1/3			0/2		1/2	1/1
5		10	1/4	0/3	0/3	0/2	0/5	0/4	4/4	2/2
6	*Idem* × 3 days	6	0/5	0/4			0/6		2/2	1/1

* TAB diluted to contain 25 million typhoid bacilli in 0.5 ml or 1.0 ml for S.C. or I.V. inoculation, respectively.

† Numerator = base line titer; denominator = peak titer of each animal. Typhoid "H" titers of 0, 10, 20, etc., to 320 coded as 0, 1, 2, etc., to 6, respectively.

absorbed agglutination tests, respectively. After 20% cell booster (Table I, Exp. 4) 1 of 4 splenectomized monkeys showed significant rise in hemolysin and absorbed agglutination titer while 1 showed only hemolysin titer increase. Both controls showed significant rise in all 3 tests. Thus after boosters 4 of 8 splenectomized monkeys and 3 of 4 controls exhibited significant hemolysin titer increases; 1 of 8 and 2 of 8 splenectomized monkeys as compared to 3 of 4 and 4 of 4 controls, respectively, showed significant titer increases in agglutination with non-absorbed and absorbed serum, respectively.

The effect of 3 successive daily intravenous inoculations of 1 ml of 20% sheep cells was studied in 4 splenectomized and 2 control monkeys (Table I, Exp. 5,6). All of 4 splenectomized monkeys showed 2 to 3-tube agglutination titer rises with unabsorbed serum as compared to 4-tube rise in both controls, but only 1 of 4 showed significant rise after absorption. Hemolysin titers showed 2, 6 and 6-tube titer increases in 3 of 4 splenectomized monkeys, respectively, as compared to 4 and 6-tube titer rise in 2 controls, respectively.

In general, non-splenectomized monkeys inoculated with sheep erythrocytes exhibited peak titers 1 week after inoculation as compared to 2 weeks or later in the majority of splenectomized animals (Table I). The decline in antibody titer after the peak was not significantly different in the 2 groups during the 12-week period.

Four splenectomized monkeys were inoculated subcutaneously with 0.5 ml of tularemia vaccine 2 weeks after operation as were 2 controls. As shown in Table II, Exp. 1, all 6 monkeys reacted similarly; 4 to 5-tube agglutination titer rises were observed in both splenectomized and non-splenectomized animals 1 week after inoculation. Comparable results were observed in monkeys similarly inoculated with tularemia vaccine 6 weeks post-splenectomy (Table II, Exp. 2). The comparative antibody rise and decline were essentially similar during the 12-week period studied.

Subcutaneous inoculation of typhoid vaccine also resulted in significant rises in "H" antibody titer in splenectomized monkeys. Four splenectomized and 2 control monkeys were inoculated 10 weeks post-splenectomy with 0.5 ml of diluted typhoid vaccine containing 25 million organisms. As seen in Table II (Exp. 3), titer increases were actually greater in splenectomized monkeys; rises of 3, 4, 4 and 5 tubes, respectively, were observed whereas both control animals showed a 3-tube increase in titer.

For comparative purposes, 1 ml of typhoid vaccine containing 25 million bacilli was given intravenously to 6 splenectomized and 3 control monkeys 2 or 10 weeks post-splenectomy as shown in Table II (Exp. 4,5). Five of 6 splenectomized animals and the

3 controls showed a 4-fold or greater "H" titer rise. Titer increases were slightly lower in the former group, however. Antibody decline was similar during the 12-week period of observation. Comparable results were obtained in 2 splenectomized monkeys and 1 control receiving 3 daily intravenous injections of 25 million killed typhoid bacilli 6 weeks post-splenectomy (Table II, Exp. 6). No difference was observed between splenectomized and non-splenectomized monkeys in time of attainment of peak titer or decline in titer after the peak. The results with "O" agglutinins parallelled those observed with "H" antigen. Thus, the antibody response of splenectomized monkeys to typhoid vaccine given intravenously was significantly greater than that observed after inoculation of sheep erythrocytes (Table II, Exp. 1,2).

Discussion. Considerable data have been published on the role of the spleen in antibody production. Results have varied depending on animal used and type, dose and route of antigen. In general, results obtained, predominantly in non-primates, and in a limited number of studies in humans have supported the concept that splenectomy may diminish antibody response if antigens are given intravenously, but not subcutaneously. To our knowledge, no studies of this nature have been done in monkeys whose antibody response, anatomy and relative spleen size simulate that of man.

The results in this study of monkeys are in agreement with previous observations from this laboratory in humans(1) that antibody response after a single subcutaneous injection of tularemia vaccine is not impaired post-splenectomy. Similarly, antibody response after a single subcutaneous injection of typhoid vaccine was not depressed in splenectomized monkeys. However, intravenous inoculation of 1 ml of 2% sheep erythrocytes was followed by significant hemolysin titer rise in only 3 of 8 splenectomized monkeys as compared to all of 4 controls. This is in agreement with previous studies employing hemolysin as the antibody measure(5-9). Using the same sera in agglutination tests only 1 of 8 splenectomized monkeys and all 4 controls showed significant titer rise. After a single intravenous booster of sheep cells 8 to 12 weeks later, only 4 of 8 splenectomized monkeys showed significant hemolysin titer rise as compared to 3 of 4 normals. However, 3 daily intravenous injections of 1 ml of 20% sheep cells resulted in significant hemolysin titer increase in 3 of 4 splenectomized monkeys as well as in both controls. With the agglutination test employing unabsorbed sera, all of 4 test animals reacted similarly to the normal controls, but only 1 of 4 showed significant rise with absorbed sera. These data would suggest that with erythrocytes, given intravenously, an increased quantity of antigen is required to stimulate extra-splenic tissue to induce comparable antibody response between splenectomized and normal monkeys. Also, the type of serologic test employed may determine, in part, differences or similarities in response. On the other hand, a single intravenous inoculation of 25 million killed typhoid bacilli yielded comparable results in 5 of 6 splenectomized monkeys as compared to the 3 controls. These results were similar to those observed when the vaccine was given subcutaneously. With 3 daily intravenous injections of vaccine similar results were observed in 2 splenectomized and 1 control animal.

These data would suggest that the type of antigen as well as dose and route of inoculation may determine, in part, the antibody response derived from extra-splenic sources. Apparently red blood cells are not as effective as bacterial antigen in eliciting antibody response in splenectomized monkeys when given intravenously. Other data from this laboratory, to be reported separately, have demonstrated comparable antibody responses in splenectomized and normal monkeys infected with intravenous challenge of streptococci and after aerosol challenge with streptococci, influenza virus and *R. rickettsii.*

Summary. Splenectomized monkeys showed antibody responses similar to normal monkeys after subcutaneous inoculation with tularemia and typhoid vaccines and after

intravenous typhoid vaccine. Impaired antibody response followed a single intravenous inoculation of sheep erythrocytes as well as after a single booster, but not after 3 daily intravenous injections.

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Erythropoietic Activity in Human Urine Concentrate.* (29361)

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Urine, concentrated by flash evaporation, has been used by several investigators to stimulate erythropoiesis in assay animals(1, 2,3). The purpose of this work is to characterize the activity of erythropoietin prepared from urine by flash evaporation and ethanol precipitation.

Materials and methods. All reagents used for preparative and analytical procedures were of reagent grade except that the ethyl alcohol which was U.S.P. Glucagon from Eli Lilly Co. and Thytropar from Armour Pharmaceutical Co. were used to characterize dialyzing membranes.

Urines, collected from patients with refractory anemia(2), were dialyzed against distilled water and then concentrated 10 to 20 times by flash evaporation at 37°C with a continuous flow apparatus. The dialyzing tubing was 1⅞ inch (inflated diameter) regenerated cellulose. The sediment from the urine concentrate was removed by centrifugation.

The alcohol precipitation of proteins was under controlled conditions as recommended by Cohn(4) and adapted by Pearsall and Chanutin(5). The urine concentrate was adjusted to pH 5.0 with an acetate buffer, and fractions were obtained at concentrations of 50 and 80 volumes per cent ethanol and at varying ionic strengths. All precipitates were dissolved in distilled water, dialyzed against distilled water, and assayed for erythropoietic activity.

Dialysis experiments were done on urine concentrates using two techniques. Ten to twenty ml aliquots of urine concentrate were dialyzed to equilibrium against equal volumes of distilled water for a period of 4-7 days at 2°C. The dialyzate was stirred thoroughly several times during each day. Erythropoietic activity was measured on the urine concentrate, the dialyzand and the dialyzate. The second technique differed in

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