cline markedly from weaning time to about 4 months of age. In the present study it was shown that the pituitary and plasma levels of TSH were low at weaning time and gradually increased until 80 or 95 days of age, then showed a slight decline. In order to explain the observations of a declining TSR associated with an increase in plasma TSH, it has been suggested that (1) the sensitivity of the thyroid gland to TSH decreases with age, (2) the $t_{1/2}$ of TSH may decrease with increasing age, and (3) the secretion of L-T_3 in the immature rat may be high and decline with age.

1. Narang, G. D., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1966, v121, 203.
2. Levey, H. A., Endocrinology, 1962, v71, 763.
3. Levey, H. A., Cheever, E., Roberts, S., ibid., 1956, v58, 420.
4. Read, C. H., Bryan, G. T., Recent Progr. Hormone Res., 1960, v16, 187.
5. Wide, L., Gemzell, C. A., Acta Endocrinol., 1960, v35, 261.
6. Pascasio, F. M., Selenkow, H. A., Endocrinology, 1962, v71, 254.
7. Selenkow, H. A., Saravis, C. A., Garcia, A. M., Acta Endocrinol., 1966, v51, 32.
8. D'Angelo, S. A., Endocrinology, 1961, v69, 834.
9. Bakke, J. L., Lawrence, N. L., ibid., 1962, v71, 43.
10. Stavitsky, A. B., J. Immunol., 1954, v72, 360.
11. Pitt-Rivers, R., Rall, J. E., Endocrinology, 1961, v68, 309.
12. Bauman, T. R., Turner, C. W., ibid., 1955, v76, 537.

Received June 16, 1966. P.S.E.B.M., 1967, v124.

Effect of Splenectomy on Renal Arteriovenous Reduction in Hemoglobin Concentration in the Anesthetized Dog.* (31832)

J. SANTOS-MARTINEZ AND ROGER M. REINECKE
School of Medicine, University of Puerto Rico, San Juan, P. R.

In a preceding study it was found in the anesthetized dog that the kidney apparently removed something measured as cyanmethemoglobin from the blood passing through it (1). In the investigation presented here the effect of splenectomy on this finding was studied.

Procedure. The apparent concentration of hemoglobin measured as carbon monoxide hemoglobin, acid hematin, and cyanmethemoglobin in the renal artery and vein was observed first in the nonsplenectomized and then in the splenectomized dog. In general the procedure with some exceptions was the same as that followed in the second group of experiments of the preceding study. Adult mongrel dogs of both sexes were anesthetized with sodium pentobarbital given intravenously. An initial dose of 20 mg/kg was supplemented with additional doses of 5 mg/kg as needed to maintain anesthesia throughout the experimental period of some hours. Polyethylene catheters were implanted in the left renal artery and the left renal vein with their open tips within the renal hilum through a ventral laparotomy. The left gonadal vein was ligated. The catheters were led out of the body through stab wounds and the laparotomy was closed with towel clamps. When the spleen was removed, this was done just before the catheters were implanted. The subjects were heparinized some minutes after the laparotomy was closed. Samples of about 1 ml of blood were drawn alternately in close succession instead of simultaneously from the arterial and venous catheters. In the nonsplenectomized animals the interval between samples was about 60 seconds and in the splenectomized animals about 30 seconds. Two to 5 ml of blood were drawn and discarded just before a sample was to be taken to clear the catheter of stagnant blood. Duplicate subsamples of each sample were analyzed for hemoglobin as carbon monoxide hemoglobin, as acid hematin, and as cyanmethemoglobin as in the second group of

* Supported in part by grant No. H-3053 (C_2) from Nat. Inst. Health, USPHS.

TABLE I. Renal Arteriovenous Reduction in Hemoglobin Concentration Measured as Carbon Monoxide Hemoglobin, as Acid Hematin, and as Cyanmethemoglobin.

Categories of differences	No. of cases	Variance $(g\ \%)^2$	P from the distribution of F*	Mean reduction† ± S.E. g %	P from the distribution of t
Nonsplenectomized subjects					
Carbon monoxide hemoglobin					
AV pairs	27	.4007	.05–.01	.08 ± .12	.50–.40
VA "	25	.2098	>.20	.04 ± .11	.80–.70
Subsamples	54	.3347			
Acid hematin					
AV pairs	27	.1065	<.001	–.04 ± .06	.50–.40
VA "	25	.1265	<.001	.02 ± .07	.80–.70
Subsamples	54	.0269			
Cyanmethemoglobin					
AV pairs	27	.8654	<.001	.39 ± .16	.05–.02
VA "	25	.5824	.01–.001	.29 ± .14	.05–.02
Subsamples	54	.2322			
Splenectomized subjects					
Carbon monoxide hemoglobin					
AV pairs	37	.0840	<.001	–.002 ± .10	>.90
VA "	37	.0183	>.20	.04 ± .05	.50–.40
Subsamples	74	.0240			
Acid hematin					
AV pairs	37	.1455	<.001	.06 ± .07	.50–.40
VA "	37	.0652	<.001	.06 ± .04	.20–.10
Subsamples	74	.0140			
Cyanmethemoglobin					
AV pairs	37	.0629	.01–.001	.05 ± .04	.30–.20
VA "	37	.0430	.05–.01	–.02 ± .03	.60–.50
Subsamples	74	.0236			

* F = variance of pairs indicated/variance of corresponding subsamples.
† Negative value of the mean indicates an arteriovenous increase.

experiments of the preceding study. For the purpose of calculating arteriovenous changes in hemoglobin concentration, each arterial sample was paired with the next following venous sample (AV pairs) and each venous sample was paired with the next following arterial sample (VA pairs). From 5 to 13 such pairs were obtained from each of 7 nonsplenectomized subjects and from 13 to 15 from each of 5 splenectomized subjects.

Results and discussion. The results are summarized in Table I. The results for the experiments in which the spleen was not removed in general are in accord with the results of the second group of experiments of the preceding study. A significant renal arteriovenous reduction in the apparent concentration of hemoglobin measured as cyanmethemoglobin was found in both the AV and VA pairs under conditions such that there was no appreciable reduction in the concentration of hemoglobin measured as carbon monoxide hemoglobin or as acid hematin. In the experiments in which the spleen was removed, the renal arteriovenous reduction in the concentration of hemoglobin measured as cyanmethemoglobin largely disappeared. The renal arteriovenous reduction in the concentration of hemoglobin measured as cyanmethemoglobin is significantly greater for the AV and also for the VA pairs in the experiments in which the spleen was not removed than in those in which it was. P = 0.02-0.01 from the distribution of t in both cases.

The simplest explanation of these results would appear to be that the presence of the spleen leads to a change in the blood measurable as cyanmethemoglobin that in turn is promptly reversed or eliminated by the kidney. The prompt action of the kidney would be explicable in terms of the large renal blood flow, in view of which it might also be expected that if, as seems likely, the change in the blood occurred within the spleen and were manifest as a splenic arteriovenous increase in the apparent concentration of hemoglobin measured as cyanmethemoglobin, this increase would be several-fold greater than the observed renal arteriovenous decrease. Such a larger splenic increase might well be of considerable help in the elucidation of what is changed and how it is changed. As pointed out in the previous report apropos somewhat more extensive data, the renal decrease is several-fold too large to represent directly a continuing, irreversible change in the hemoglobin molecule in relation to current concept in regard to survival time of the erythrocyte. Otherwise it has been recognized for some time that the spleen produces changes in the erythrocyte(2,3,4) and more recently that nephrectomy may lead to hemolysis(5,6) and that the kidney may evoke erythropoiesis(7,8).

Summary. A renal arteriovenous reduction in hemoglobin concentration measured as cyanmethemoglobin observed in the anesthetized dog largely disappeared after removal of the spleen. It is surmised that the presence of the spleen leads to changes in the blood that are reversed or otherwise eliminated by the kidney.

1. Santos-Martinez, J., Reinecke, R. M., Proc. Soc. Exp. Biol. and Med., 1966, v121, 1049.
2. Cunningham, R. S., Sabin, F. R., Doan, C. A., ibid., 1923, v21, 326.
3. Krumbhaar, E. B., Physiol. Rev., 1926, v6, 160.
4. Higgins, G. M., Ann. Rev. Physiol., 1941, v3, 283.
5. Muirhead, E. E., Jones, F., Stirman, J. A., Lesch, W., Am. J. Physiol., 1953, v173, 371.
6. Muirhead, E. E., Jones, F., Blood, 1963, v22, 272.
7. Suki, W., Grollman, A., Am. J. Physiol., 1960, v100, 629.
8. Naetz, J. P., Blood, 1960, v16, 1770.

Received July 5, 1966. P.S.E.B.M., 1967, v124.

Effect of Increased Urine Excretion on Renal Arteriovenous Reduction in Hemoglobin Concentration in the Anesthetized Dog.* (31833)

J. SANTOS-MARTINEZ AND LUIS A. VÁZQUEZ
(Introduced by Roger M. Reinecke)
School of Medicine, University of Puerto Rico, San Juan, P.R.

A renal arteriovenous reduction in the concentration of whatever is measured as cyanmethemoglobin was observed in a preceding study(1). While it seemed likely that this reduction would tend to be eliminated or even reversed by the increased removal of water from the blood flowing through the kidney during diuresis, other possibilities could not be excluded *a priori*. The matter was studied in the investigation reported here by observing the renal arteriovenous difference in the apparent concentration of hemoglobin measured as cyanmethemoglobin in the anesthetized dog before and during diuresis produced by infusing an aqueous solution of mannitol.

* Supported in part by General Research Support Grant from NIH to School of Medicine.

Procedure. Seven adult mongrel dogs of both sexes weighing 10-20 kg were used as subjects. In general the same procedures were followed as in the study reported in the accompanying paper(2) in inducing and maintaining anesthesia, in implanting catheters in the renal vessels, and in taking and analyzing blood samples with the exceptions that the arterial and venous pairs of blood samples were drawn simultaneously and analyzed in terms of cyanmethemoglobin only. In addition polyethylene catheters were implanted in both ureters when the abdomen was opened for implanting the catheters in the renal vessels. The open tips of these ureteral catheters were advanced into the renal pelves, and like those in the renal vessels they were led out of the body through