plicate this measurement, for example, the catheter aperture opens into the stream of blood in the vein and is oppositely situated in the artery. As a result the kinetic energy factor is added to lateral pressure in the first case and subtracted in the second. For this reason one cannot, without velocity measurements or their equivalent, give absolute lateral pressure values. It is possible, however, that with bradycardia the pulmonary vascular pressure gradient direction is actually reversed in diastole. A slow diastolic rise in pulmonary artery pressure seen only in this case would conform with this interpretation. The maximum systolic pulmonary arterial pressure is greatly increased in bradycardia.

In one case the cardiac output was measured by the direct Fick method before, during and immediately after vagal stimulation. The values obtained for the cardiac outputs were 3.2, 2.0 and 2.4 L. per min. in the 3 situations in the order named. The systemic venous pressures rose 2-6 cm H_2O during the vagal stimulation periods and the mean femoral arterial pressure fell slightly although the pulse pressure increased.

In two experiments with acetylcholine and one with acetyl-beta-methylcholine administered in such doses as to lower the heart rate to a half or less of the control value similar changes in pulmonary vascular pressures were recorded.

The observations recorded herein are entirely in harmony with predictions from classical hemodynamics. They are being reported primarily because they have a bearing on the mechanism of production of pulmonary congestion and edema, in states of bradycardia. In one respect the effects of acute vagal stimulation appear to differ from the results when comparable bradycardia occurs gradually after increasing the intracranial pressure. In the latter case the pulmonary artery pressure rises, in contrast to the fall reported herein for acute vagal bradycardia. It should be noted that when vagal stimulation was continued for 10 minutes, the bradycardia being maintained, the pulmonary artery pressure rose to values above the control level. The pulmonary venous pressure remains elevated. The mechanism of the delayed rise in pulmonary artery pressure has not been analyzed. It is obvious from the observations reported here that simple bradycardia can account for the elevation in pulmonary venous pressure seen when cardiac slowing results from elevations in intracranial pressure. Since in the latter situation the ensuing pulmonary edema is correlated with the pulmonary venous pressure[3] one can also link the genesis of the edema with bradycardia.

Summary. The bradycardia produced by vagal stimulation and by the administration of acetylcholine or acetyl-beta-methylcholine resulted in an elevation of mean pulmonary venous pressure and a small fall in mean pulmonary artery pressure. These observations are discussed in relation to the genesis of pulmonary edema in situations in which bradycardia occurs.

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17077. Production of Anemia in Swine Fed Purified Diets and Sulfasuxidine.*

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In 1946 it was reported from this laboratory[1] that a pig maintained on a diet in which purified casein (Borden's Labco "vitamin-free") was substituted for crude casein (Sheffield's New Process) and to which 2 per cent

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1 Cartwright, G. E., Wintrobe, M. M., and Humphreys, S., *J. Lab. and Clin. Med.*, 1946, **31**, 423.

sulfasuxidine was added, failed to grow normally and developed a normocytic anemia. Following treatment with a highly purified antipernicious anemia liver extract, growth was resumed and, after a definite although not marked reticulocytosis, the blood returned to normal. This observation has been confirmed by Cunha *et al.*[2] However, Welch *et al.*[3] fed a similar diet to two 63 day old pigs and failed to observe either retardation of growth or anemia. Cunha *et al.* reported that the development of the anemia was prevented by the administration of pteroylglutamic acid and, to a lesser extent, by antipernicious anemia liver extract.

More recently it has been demonstrated in this laboratory[4,5] as well as by Welch and Heinle,[6-8] that swine fed a similar purified casein diet supplemented with sulfasuxidine to which a folic acid antagonist is added, develop severe macrocytic anemia, leukopenia, slight thrombocytopenia and hyperplasia of the bone marrow. In the bone marrow there is an increase in immature nucleated red cells which, furthermore, resemble the megaloblasts seen in the marrow of patients with pernicious anemia. This anemia responds rapidly to the administration of pteroylglutamic acid. Purified liver extracts manifest some hemopoietic activity in such animals but the activity is considerably less than that of pteroylglutamic acid.[4,5]

The purpose of the present study was to determine if our original observation could be confirmed and, if so, whether the anemia so produced differs either morphologically or in its response to therapy, from the anemia produced in swine given a folic acid antagonist.

Experimental. A total of 75 weanling Chester-White pigs, 21 to 28 days of age were used. The animals were divided into three groups as follows:

Group	No. pigs	Type casein	Sulfasuxidine‡ %
I	12	Purified*	2
II	42	Crude†	0
III	21	"	2

All animals received the following vitamins: A, D, thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, choline and inositol. In addition, those in group II were given pteroylglutamic acid, animals in group I were given para-aminobenzoic acid and those in groups II and III were given biotin. The percentage of casein fed is indicated in Tables I and III. Full details of the experimental methods, the composition of the basal diet and the amounts of vitamins administered have been given in previous reports.[1,4,5,9]

Results. The hematologic studies in the 12 animals in group I are presented in Table I. Anemia developed in each instance. The anemia was mild (volume of packed red cells 30 to 35 ml/100 ml) in 5 pigs, moderately severe (volume of packed red cells 25 to 30) in 4 pigs, and severe (volume of packed red cells less than 25) in 3 pigs. The anemia was normochromic and in general normocytic although the mean corpuscular volume was greater than 65 $c\mu$ in 4 pigs. Differential cell counts on marrow aspirated from the sternum revealed a decrease in the leukocyte-erythroid ratio with many immature nucleated red cells present. These cells did not differ significantly in their morphologic characteristics from those observed in the marrow of

2 Cunha, T. J., Colby, R. W., Bustad, L. K., and Bone, J. F., *J. Nutrition*, 1948, **36**, 215.

3 Welch, A. D., Heinle, R. W., Sharpe, G., George, W. L., and Epstein, M., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 364.

4 Cartwright, G. E., Fay, J., Tatting, B., and Wintrobe, M. M., *J. Lab. and Clin. Med.*, 1948, **33**, 397.

5 Cartwright, G. E., Tatting, B., Ashenbrucker, H., and Wintrobe, M. M., *Blood*, 1949, **4**, 301.

6 Heinle, R. W., Welch, A. D., George, W. L., Epstein, M., and Pritchard, J. A., *J. Lab. and Clin. Med.*, 1947, **32**, 1398.

7 Welch, A. D., Heinle, R. W., Pritchard, J. A., and Solis, H., *Fed. Proc.*, 1948, **7**, 300.

8 Heinle, R. W., Welch, A. D., and Pritchard, J. A., *J. Lab. and Clin. Med.*, 1948, **33**, 1647.

* Either Borden's Labco "vitamin-free" casein or Sheffield's special alcohol extracted casein.[1]

† Sheffield's New Process (Crude) Casein.

‡ Kindly furnished by Sharp and Dohme, Inc., Philadelphia, Pa., through the courtesy of Dr. W. A. Feirer.

9 Wintrobe, M. M., Miller, J. L., and Lisco, H., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 377.

TABLE I.
Hematologic Observations in Pigs Receiving Purified Casein and Sulfasuxidine (Group I).

Pig No.	Casein in diet, %	Day of exper.	RBC millions per c.mm.	Hgb. gm, %	VPRC ml/100 ml	MCV, $c\mu$	MCH, $\gamma\gamma$	MCHC, %
9–31	18	135	3.70	6.6	21.0	57	18	31
32	18	206	3.93	9.4	28.0	71	24	34
52	26	136	5.19	10.4	30.5	59	19	33
53	26	85	5.64	9.0	30.0	54	16	33
66	12	130	3.57	8.1	24.5	69	23	33
67	12	130	4.67	10.1	30.0	64	22	34
69	18	130	3.03	6.9	19.5	64	23	35
10–66	26	190	4.68	12.4	34.8	74	26	36
68	26	190	4.15	11.3	32.0	77	27	35
69	26	139	4.52	9.1	28.2	62	20	32
71	26	153	4.60	9.2	28.0	63	21	33
82	26	94	5.13	9.9	29.6	58	19	33

VPRC refers to volume of packed red cells; MCV refers to mean corpuscular volume; MCH refers to mean corpuscular hemoglobin; MCHC refers to mean corpuscular hemoglobin concentration.

TABLE II.
Response of the Anemia in Group I to Pteroylglutamic Acid (PGA) and Liver Extract Therapy.

Pig No.	Substance	Dose I.M.	Before therapy		After therapy			
					Retic peak		Vol. pkd. RBC	
			VPRC, ml/100 ml	Retics, %	%	day	ml/100 ml	day
9-31	PGA	50 mg	24.5	0.6	5.4	3	37.0	17
32	PGA	50 mg	28.0	0.8	3.6	4	42.5	10
66	PGA	50 mg	24.5	1.0	16.0	5	44.0	14
67	PGA	50 mg	30.0	1.0	11.8	3	40.0	18
69	PGA	50 mg	19.5	0.2	8.0	7	39.5	14
10-66	PGA	20 mg	35.0	2.0	6.0	7	45.5	14
68	Liver extr.*	150 u	32.0	0.8	5.0	2	48.0	28
69	" "	150 u	28.2	2.0	8.0	5	35.0	14
69	PGA	20 mg	31.0	0.8	12.0	5	45.5	21
71	Liver extr.*	75 u	27.0	2.2	6.0	5	37.0	14
71	PGA	20 mg	35.0	1.0	14.0	5	42.0	11

* Parke, Davis and Company, 15 U.S.P. units per ml.
u refers to U.S.P. units of liver extract.

pigs receiving a crude methyl folic acid antagonist.[4,5] However, in general the changes were not as marked.

The results of therapy with pteroylglutamic acid and with liver extract are presented in Table II. The administration of pteroylglutamic acid resulted in a significant reticulocytosis and a rise in volume of packed red cells to normal. The administration of liver extract was followed by a slight reticulocytosis and a significant although suboptimal rise in volume of packed red cells. In two of the pigs so treated the administration of pteroylglutamic acid resulted in a second reticulocytosis and a further increase in the volume of packed red cells.

The hematologic studies in the animals in groups II and III are presented in Table III where are compared the volumes of packed red cells and the mean corpuscular volumes of pigs fed various proportions of crude casein with and without pteroylglutamic acid and sulfasuxidine. It is clear from these data that, like the animals receiving purified casein (group I, Table I), the inclusion of sulfasuxidine in a pteroylglutamic acid-"free" crude casein diet also resulted in the development of significant anemia (group III). The anemia was comparatively more severe in the pigs fed casein at a 10 per cent level than in those receiving a diet containing 26 per cent casein. In animals given a folic

TABLE III.
Hematologic Observations in Pigs Receiving Crude Casein.
(Groups II and III). Mathematical Averages and Ranges.

Dietary casein, %	Group II			Group III		
	No. pigs	VPRC, ml/100 ml	MCV, c.μ.	No. pigs	VPRC, ml/100 ml	MCV, c.μ.
26	10	42.0 (39-47)	53 (51-63)	5	37.3 (31-41)	58 (57-63)
18	10	37.1 (34-41)	56 (50-62)	10	29.7 (25-37)	58 (51-62)
10	22	35.7 (34-40)	55 (49-65)	6	24.6 (21-32)	56 (51-63)

Group II received pteroylglutamic acid and no sulfasuxidine.
Group III received no pteroylglutamic acid but were given sulfasuxidine.
VPRC refers to volume of packed red cells.
MCV refers to mean corpuscular volume.
Figures in parentheses represent range.

acid antagonist, it has also been found that pteroylglutamic acid deficiency develops more rapidly on a diet low in protein.[5]

There were certain dissimilarities between the anemia produced on a crude casein diet and the anemia produced on the diet containing purified casein. No anemia (volume of packed red cells greater than 35 ml/100 ml) was present in 6 (29 per cent) of the pigs fed a crude casein diet. The anemia when it developed was mild (volume of packed red cells, 30 to 35) in 4 pigs (23 per cent), moderately severe (volume of packed red cells, 25 to 30) in 8 (38 per cent), and severe (volume of packed red cells less than 25) in only 2 (10 per cent). Thus the anemia was not produced as consistently, nor was it as severe, as in pigs fed the purified casein. It developed somewhat more slowly and when present, was normocytic. Furthermore, after the development of anemia in the animals fed crude casein in each instance reticulocytosis appeared spontaneously and this was followed by the complete disappearance of the anemia (Fig. 1). The reticulocytosis was gradual in its development and persisted for several weeks, giving a low, flat curve. A sharp peak, such as follows the administration of pteroylglutamic acid (Fig. 1), was not observed. The degree of reticulocytosis varied from 6 to 23 percent and usually reached 10 to 15 per cent.

Summary. Pigs maintained on a diet in which purified casein was substituted for crude casein and to which 2 per cent sulfasuxidine was added developed anemia which responded partially to purified liver extract and completely to pteroylglutamic acid. This anemia resembles that which has been produced in pigs given a folic acid antagonist.

Pigs maintained on a diet containing crude casein and to which 2 per cent sulfasuxidine was added developed less pronounced anemia which disappeared spontaneously.

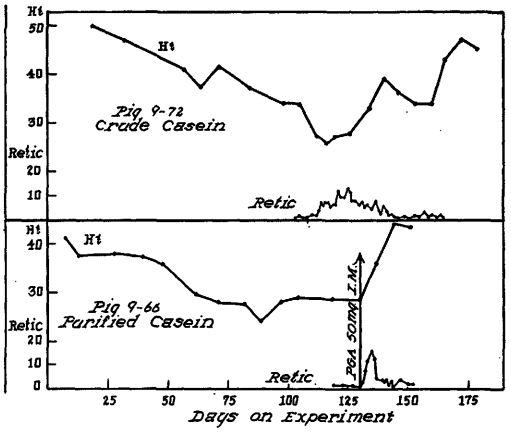

FIG. 1.
Pig 9-72 (Group III) received crude casein (12%). Note the development of anemia and the spontaneous reticulocytosis with relief of the anemia. Pig 9-66 (Group I) received purified casein (12%). Note the persistent anemia and the rapid response with a sharp reticulocyte peak which followed the administration of pteroylglutamic acid.

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