

oxygen pressure; at 53 mm a slight depression of secretion appears. This apparent diminution, however, is not statistically significant.

The intestinal glands are seen to be rather highly resistant to anoxia, probably indicating that they have a low energy requirement for secretion. The questionable depression of secretion at 53 mm Hg oxygen pressure is slight at best; the glands are still secreting quite well. Not all anesthetized dogs will survive this degree of anoxia for 20 minutes.

*Summary.* Intestinal secretion in dogs was found to be resistant to anoxia. At a partial pressure of oxygen of 80 mm Hg (approximate altitude of 18,000 ft.) there was no effect, and at 53 mm Hg (28,000 ft.) only a mild depression of secretion, which was not statistically significant. This indicates a low energy requirement for intestinal secretion.

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**Effects of Vitamin C Deficiency and Diphtheria Toxin on Cellular Blood Constituents of Guinea Pig.\***

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The literature contains many reports concerning the anemia associated with scurvy in both humans and experimental animals.<sup>1-10</sup> There is general agreement that a continued lack of vitamin C in the diet causes a progressive decrease in erythrocytes and hemoglobin, and that the anemia becomes severe only during the later stages of a deficiency. The cause of the anemia is not well understood, however.

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Considerable evidence<sup>5</sup> indicates that it is due to cytological changes in the bone marrow, resulting in a retarded production of erythrocytes, rather than to a specific deficiency of hemoglobin or an excessive destruction of erythrocytes.

There is apparent disagreement concerning the effects of scurvy on the leucocyte count of the blood. Using guinea pigs, von Euler and Malmberg,<sup>8</sup> and Shimada<sup>7</sup> have reported a decrease in leucocytes accompanying the depletion of vitamin C, but Liotta<sup>3</sup> and Aszodi<sup>10</sup> have reported an increase under similar conditions.

While studying the effects of vitamin C depletion<sup>11</sup> and diphtheria toxin injections<sup>12</sup> on the glucose tolerance of guinea pigs, we were afforded an opportunity of studying the parallel changes in cellular blood constituents, with the kind assistance of Miss Sarita Bailey, of the Children's Hospital.

Essentially, we observed that a decrease in hemoglobin and erythrocytes, and a slight increase in leucocytes accompanied the development of scurvy. In the experiments with toxin we observed that the level of vitamin C intake influenced the effects of the toxin, as measured by changes in the cellular blood picture.

The results supplement our finding<sup>12</sup> that the effects of diphtheria toxin on the glucose tolerance of guinea pigs are more severe when the vitamin intake has been low. Numerous reports have shown that the vitamin plays a rôle in decreasing the severity of infection.<sup>13-17</sup> The increased protection afforded by a high vitamin intake is due apparently to the general physiological benefits of a better state of nutrition of the animals, rather than to a direct chemical inactivation of the toxin by the vitamin.<sup>18</sup> We have observed acidic inactivation of the toxin *in vitro*, but neutral, buffered solutions of the vitamin failed to exert a significant effect upon dilute solutions of the toxin.

*Experimental.* Guinea pigs weighing approximately 300 g were fed the basal diet of Sherman and collaborators<sup>19</sup> supplemented with cod liver oil, yeast (2%) and a solution of crystalline vitamin C until they had reached 450 to 600 g in weight. The animals were all of

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<sup>18</sup> Sigal, A., and King, C. G., *J. Pharm.*, 1937, **59**, 468.

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TABLE I.  
Effects of Vitamin C Deficiency and Diphtheria Toxin Injections on Cellular Constituents of Blood of Guinea Pigs.  
All determinations were made on the 20th or 21st experimental day, except for the 10 days depletion group. Toxin injections, 0.2 M.L.D. each, were made on the 10th and on alternate succeeding days (5 injections).

Group	No. of animals	Hemoglobin % Sahli	Erythrocytes, millions	Leucocytes, thousands	Polymorpho-nuclear leucocytes	Lymphocytes	Monocytes	Eosinophiles	Basophiles	Nucleated red cells per 100 leucocytes
3 mg/day, no toxin	16	87 (80-96)*	4.4 (4.0-4.9)	7.8 (6.1-10.8)	45 (30-57)	50 (38-61)	1.5 (0-4)	3.0 (0-8)	0.7 (0-4)	
3 mg/day, and toxin injections	10	79 (69-88)	4.0 (3.6-4.2)	9.7 (6.2-15)	45 (36-60)	51 (36-61)	2.8 (0-5)	2.0 (0-8)		
0.5 mg/day, no toxin	9	77 (70-84)	3.9 (3.5-4.2)	8.4 (6.1-11.2)	45 (30-62)	49 (36-60)	3.0 (0-6)	2.2 (0-14)	.8 (0-2)	
0.5 mg/day, and toxin injections	7	63 (45-80)	3.3 (2.4-4.2)	18.6 (12-34)	64 (52-78)	30 (18-44)	4.0 (2-8)	2.3 (0-8)		5† (0-24)
10 days depletion, no toxin	25	82 (69-90)	4.1 (3.5-4.6)	8.4 (6.1-15.5)	45 (26-54)	46 (32-62)	4.4 (0-8)	3.0 (0-12)	.5 (0-2)	
20 days depletion, no toxin	10	58 (22-78)	3.0 (1.5-3.9)	9.4 (7.5-12.2)	46 (32-57)	46 (34-70)	5.7 (2-8)	1.2 (0-4)	.4 (0-2)	75† (0-600)
20 days depletion, and toxin	6	49 (29-65)	2.8 (1.6-3.5)	17.5 (6.9-25.2)	68 (43-79)	27 (18-42)	3.3 (1-7)	2.3 (0-8)		81† (17-300)

\* Extreme values for individual animals are given in parentheses.

† Moderate and marked chromatophilia, anisocytosis and poikilocytosis.

‡ Marked chromatophilia, anisocytosis and poikilocytosis.

approximately the same age. It had been found advisable to use fairly large animals for the sugar tolerance tests, to facilitate blood sampling.

*Toxin.* The diphtheria toxin was carefully standardized on an M.L.D. basis (238 M.L.D. per cc) with pigs weighing 250 g. Toxin dilutions in sterile physiological saline were standardized so that 1 cc of solution contained 1 M.L.D. of toxin. Injections were made subcutaneously.

*Blood samples.* The erythrocyte, leucocyte and differential counts were made in the usual manner upon blood samples obtained from a marginal ear vein.

*Vitamin C depletion.* Table I shows the effect of vitamin C depletion alone on the cellular blood constituents of guinea pigs that had been receiving 3.0 mg of vitamin daily for 20 days. The control determinations were made a few days before starting the depletion. During the 10-day depletion period there was a slight decrease in erythrocytes and hemoglobin and a slight increase in leucocytes. Between the 10th and 21st days of depletion the anemia became severe, with a parallel decrease in both hemoglobin and red blood cells and a further slight increase in leucocytes. Nucleated red cells and abnormal cell forms appeared during the onset of severe scurvy. All the animals began to lose weight and show signs of scurvy between the 10th and 15th days of depletion.

Animals receiving 0.5 and 0 mg of vitamin C per day were given 5 injections of 0.2 M.L.D. of toxin each. Injections were made on the 10th experimental day and at 2-day intervals thereafter. The determinations were made on the 20th or 21st experimental day. The data for these animals compared with control animals receiving the same amount of vitamin but no toxin are also given in Table I. The group receiving toxin and 3.0 mg of vitamin daily showed a moderate reduction in hemoglobin and erythrocytes and a moderate increase in leucocytes. The groups receiving toxin and 0.5 or 0 mg of vitamin C showed a greater drop in hemoglobin and red blood cells and a larger increase in leucocytes than shown by the 3.0 mg group. The differential counts of the toxin-treated, low-vitamin groups showed an increase in polymorphonuclear leucocytes, a decrease in lymphocytes, and the presence of many nucleated red cells and abnormal cell forms.

It is evident that the animals receiving a higher intake (3.0 mg) of vitamin C were able to withstand the effects of the toxin to a greater degree. The distinctly sub-optimum intake of vitamin (0.5 mg) was sufficient, however, to permit nearly normal growth and freedom from external signs of scurvy.

The results of the present investigation supplement, and in a way, confirm, the earlier findings in our laboratory concerning the lowered resistance against diphtheria toxin that characterizes a low intake of vitamin C. Tooth structure<sup>20</sup> and glucose tolerance<sup>12</sup> have been considered in the earlier papers.

*Conclusions.* Accompanying the anemia of scurvy in guinea pigs there was a progressive decrease in both hemoglobin and erythrocytes and a slight increase in leucocytes.

Guinea pigs receiving 3.0 mg of vitamin C per day and given subcutaneous injections of diphtheria toxin showed less disturbance in their blood cell count than similarly injected animals receiving 0.5 mg or less of the vitamin per day. The changes in the low-vitamin, toxin-injected groups were characterized by chromatophilia, anisocytosis and poikilocytosis.

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### Antihormone Effects in Pancreatic Diabetes.

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Collip and Anderson<sup>1</sup> observed that during the prolonged injection of anterior pituitary extract (thyrotropic) its effect upon the metabolic rate was not sustained. They also noted<sup>1, 2</sup> that the serum of refractory animals inhibited the response of normal animals to thyrotropic hormone. Similar inhibitory effects have been observed in the case of the gonadotropic hormone. The relation of such antagonistic principles to species has been further studied.<sup>3, 4, 5</sup>

Relatively few reports are available on the development of principles which antagonize the diabetogenic action of the anterior pituitary, although refractoriness has long been recognized. Collip<sup>6</sup> re-

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<sup>3</sup> Thompson, K. W., and Cushing, H., *Proc. Roy. Soc. (London)*, series B, 1937, **121**, 501.

<sup>4</sup> Thompson, K. W., *Proc. Soc. Exp. Biol. and Med.*, 1937, **35**, 637.

<sup>5</sup> Anderson, E., and Evans, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 797.