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Effect of Insulin on Plasma Level and Excretion of Vitamin C.*

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In studying the metabolism of vitamin C in normal and depancreatized dogs, the urinary excretion of the vitamin was found to be strikingly reduced in the diabetic animal. The diets in both

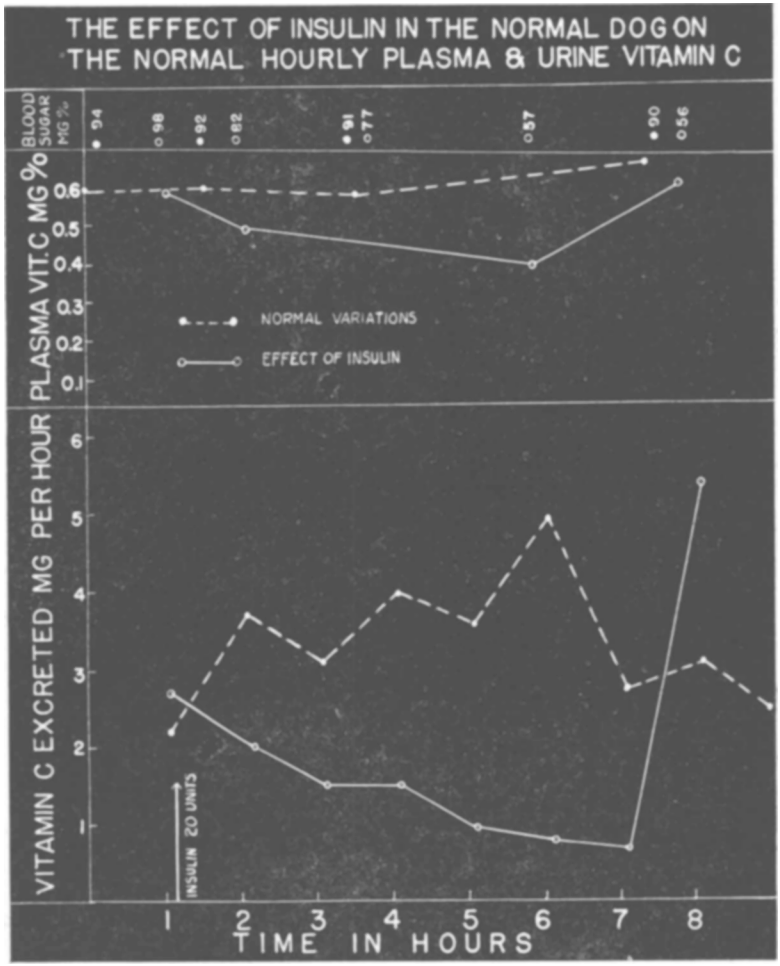

CHART 1.

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groups of animals were totally devoid of vitamin C; dried meat powder was used in place of raw beef. The dogs were kept in individual metabolism cages and the urines were collected by catheter at the start and end of each 24-hour period. Urines voided between catheterizations were collected in dark bottles containing sulphuric acid and hydroxyquinoline to prevent the destruction of the urinary ascorbic acid. Fasting bloods were taken for the determination of the plasma vitamin C.[1] Urinary vitamin C was determined by the method of Evelyn.[2] The depancreatized dogs received insulin twice daily in amounts which allowed a moderate daily excretion of sugar.

The 6 normal dogs excreted an average of 20.6 mg of vitamin C per kg of body weight in 24 hours. The fasting plasma levels of vitamin C varied from 0.55 to 0.94 mg %. The 7 diabetic dogs maintained on insulin excreted on an average only 3.6 mg of vitamin C per kg of body weight in 24 hours. The fasting plasma levels varied from 0.33 to 0.71 mg %.

The influence of injected insulin on the plasma level and excretion of vitamin C was studied in a series of acute experiments in both the normal and depancreatized dog. Control experiments were run on each dog to ascertain the hourly plasma variation and urinary excretion of vitamin C. Over a period of 8 hours the plasma levels of vitamin C in the normal dog varied from 0.58 to 0.66 mg % and

TABLE I.
Average 24-hour Excretion of Vitamin C and Fasting Plasma Levels in Normal and Depancreatized Dogs on Insulin.

Dog No.	Wt, kg	No. daily observations	Urinary excretion vitamin C in mg daily Avg	S.D.	Urinary excretion per kg Body wt	Fasting plasma levels vitamin C mg % No. obser.	Avg	S.D.
207	15.75	13	304	±32.1	19.3	5	0.55	±.09
211	12.25	17	189	±20.6	15.4	8	0.65	±.07
197	11.25	10	232	±17.0	20.6	7	0.62	±.14
193	9.0	21	163	±18.3	18.1	13	0.63	±.08
215	11.75	1	300		25.5	1	0.89	
218	**13.0**	**6**	**319**		**24.5**	2	0.94	
			Diabetic Dogs.*					
192	9.75	25	14	± 6.5	1.4	9	0.45	±.03
214	6.75	5	8	± 1.6	1.2	2	0.41	
216	8.25	5	16	± 2.0	1.9	2	0.33	
170	12.50	15	71	±21.0	5.9	5	0.49	±.12
211	10.50	13	81	± 9.0	7.7	6	0.71	±.05
215	10.50	11	29	±11.0	2.8	5	0.66	±.12
208	11.50	5	52	±13.0	4.5	2	0.66	

*The depancreatized dogs received insulin twice daily.

[1] Mindlin, R. L., and Butler, A. M., *J. Biol. Chem.*, 1938, **122**, 673.

[2] Evelyn, K. A., Malloy, H. T., and Rosen, C., *J. Biol. Chem.*, 1938, **126**, 655.

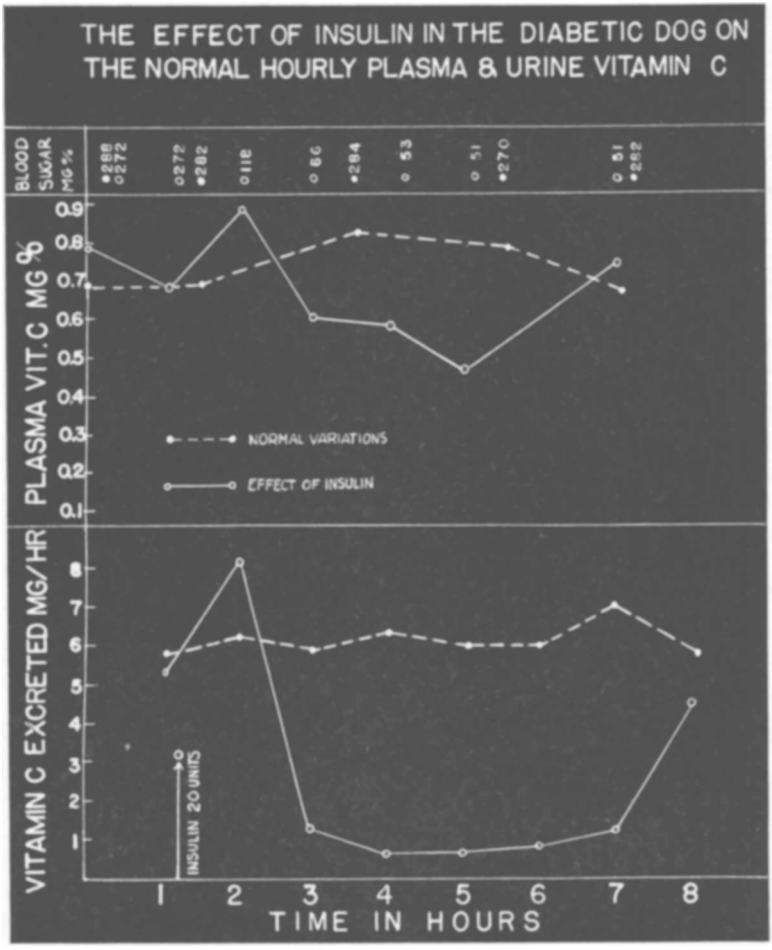

CHART 2.

the urinary excretion varied from 2.16 to 4.92 mg per hour (Chart 1). In the diabetic dog the plasma levels of vitamin C varied from 0.69 to 0.83 mg % and the urinary excretion from 5.70 to 7.04 mg per hour (Chart 2). In 3 experiments in the normal dog 20 units of insulin were injected subcutaneously and at the same time a small dose of glucose was given by mouth to avoid too rapid a fall in the blood sugar. One typical experiment is shown in Chart 1. In contrast to the normal variations in plasma levels of vitamin C, following the injection of insulin there is a fall in the plasma concentration and the urinary excretion of the vitamin. The effect lasted about 6-7 hours at which time both the plasma level and the urinary excretion returned to normal.

In the diabetic dog (Chart 2) in 3 experiments the results are

similar. There was a sharp fall in the plasma level and urinary excretion of vitamin C as soon as the subcutaneously injected insulin became effective.

In additional experiments we have found that if glucose is administered intravenously at the time that the plasma vitamin C level and urinary excretion is reduced the effect can be overcome and the plasma level of the vitamin returned to normal.

Similar studies are being made at present on human normal and diabetic subjects.

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Test for the Sterility of Biologic Products.

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Standard tests for the sterility of finished vaccines or serums consist of the inoculation of 0.25 cc and 1.0 cc from the proper number of samples of the product to Smith fermentation-tubes containing infusion-broth. The tubes are heated within 5 hours before inoculation to drive off dissolved oxygen. They are then inoculated and are incubated for 7 days. The presence of weak points in the standard procedure has long been suspected.

The inherent weaknesses of the standard test are, in part, dependent upon (1) the frequent use of merthiolate which, because of its bacteriostatic effect, prevents the growth of contaminants, if any, in many instances; and (2) standard Smith tubes are not well adapted to the cultivation of anaërobes. The use of a second transfer, after 7 days, from optically clear Smith tubes to fresh Smith tubes in an endeavor to overcome the bacteriostatic action of the preservative is definitely irrational and thus unsatisfactory.[1]

The use of Brewer's medium[2] containing thioglycollate overcomes the objections given because it permits the growth of organisms in the presence of merthiolate and because it is suited to the cultivation of anaërobes. The results reported here support these views.

Tests were made with vaccines (bacterins) and serums, preserved with merthiolate, 1:5000 or 1:10,000, or with phenol, 0.5%. As contaminants 24-hour cultures of *Staph. aureus, Staph. albus, Ps. pyocyanea, B. subtilis* and *C. xerosis* were used.[3] In addition, there

[1] Marshall, M. S., and Hrenoff, A. K., *J. Infect. Dis.*, 1937, **61**, 42.

[2] Brewer, J. H., *J. Bact.*, 1940, **39**, 10.