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Influence of Ascorbic Acid of Diet on Sensitization of Guinea Pigs to Neoarsphenamine.

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Frei reported[1] that he was able to sensitize the skin of guinea pigs to neoarsphenamine. One of us (S) observed[2] a high degree of variation in the response of different series of guinea pigs to this type of sensitization. Mayer and Sulzberger[3] concluded that the composition of the diet was of fundamental significance, for, the animals receiving summer fodder were resistant to sensitization (only 0 to 12% becoming sensitized), whereas animals receiving winter fodder were sensitizable 75 to 100%. The "seasonal" difference in the diets depended upon the inclusion or omission of greens. Sensitization of guinea pigs to paraphenylene diamine as well as their reaction to infection with a virulent strain of tubercle bacilli have been reported to be similarly influenced by diet.[3, 4]

Our attention was directed to the vitamin C content of the rations. When pure crystalline vitamin C became available, it was decided to use the synthetic product* as the source of vitamin C instead of citrus or tomato juice.

Young guinea pigs weighing approximately 250 gm. were placed upon the scorbutogenic diet described by Demole,[5] consisting of 2 kilos oat flakes and one kilo dried whole milk (previously heated for 2 hours at 120°C.) made into cakes with the aid of 6 egg whites and baked on a greased pan for 20-25 minutes. 200 mg. per week of cod liver oil were fed each guinea pig and a small amount of dried hay supplied. After 10 to 15 days on this diet, the negative control animals commenced to lose weight and showed early signs of scurvy. Three to 4 weeks later they died and at autopsy further manifesta-

[1] Frei, W., *Klin. Wochschr.*, 1928, **7**, 1026.

[2] Sulzberger, M. B., *Klin. Wochschr.*, 1929, **6**, 253; *Arch. Dermat., and Syph.*, 1929, **20**, 669, 1930, **22**, 839; Sulzberger, M. B., and Simon, F. A., *J. Allergy*, 1934, **6**, 39.

[3] Mayer, R. L., and Sulzberger, M. B., *Arch. f. Dermat. u. Syph.*, 1931, **163**, 245; Sulzberger, M. B., and Mayer, R. L., *Arch. Dermat. and Syph.*, 1931, **24**, 537.

[4] v. Engel, P., *Arch. f. Dermat. u. Syph.*, 1933, **167**, 279.

* l-Ascorbic acid in the form of Redoxon manufactured by Hoffmann-LaRoche, Inc., to whom we are indebted for a liberal supply of the product.

[5] Demole, V., *Z. f. Vitaminforschung*, 1934, **3**, 89.

tations of scurvy were found. After the tenth day on this diet, the experimental animals were divided into 5 groups and fed individual daily supplements of ascorbic acid as follows: Group I, 0.25 mg.; Group II, 0.50 mg.; Group III, 0.75 mg.; Group IV, 1.50 mg.; and Group V, 2.00 mg. A fresh 1.5% solution of ascorbic acid was prepared each day by dissolving a weighed quantity of Redoxon in cooled, freshly boiled distilled water. This was administered orally by pipette. Demole and others reported that the minimum protective dose of ascorbic acid is about 0.6-0.7 mg. per day. Titration of the preparation used in each experiment by means of 2-6 dichlorophenol-indophenol indicated that the material was 100% ascorbic acid. All guinea pigs in our Groups I and II (receiving 0.50 mg. or less ascorbic acid) developed definite clinical signs of scurvy; whereas those in Groups III, IV and V (receiving 0.75 mg. or more) were practically completely protected. On the tenth day after they had begun to receive their daily supplements of ascorbic acid, the animals were injected intracutaneously in depilated areas of the right flank with 0.1 cc. of a 0.15% solution of neoarsphenamine (Metz) in sterile saline (0.15 mg. neoarsphenamine). Daily observations were made of the site of the injections. None of the animals showed an inflammatory response immediately following the injection or during the next few days. From the sixth to tenth day, the injection sites in a certain percentage of the animals "flared-up," *i. e.*, an erythematous and infiltrated papule became manifest, which in some instances reached the size of a bean and went on to central necrosis and scarring. On the twenty-eighth day, the animals were reinjected in the opposite flank with the same dose of neoarsphenamine. The reaction differed from the first in that sensitized animals showed an inflammatory reaction within 24 hours which increased in severity for a period of days. These manifestations were identical with those observed in the earlier experiments.

During the entire period of the sensitization experiment, the animals were kept on the same basal ration and the same daily supplements of ascorbic acid.

Table I records (a) intensity of the "flare-up" reaction at the site of the initial injection and the severity of the reaction following the second or test injection; and (b) the severity of the gross clinical signs of scurvy. These are correlated with the doses of ascorbic acid fed the various groups. The 6 animals that comprised the negative control group which died of acute scurvy, are not included in this table.

Distinct hypersensitivity was produced in the major number of

TABLE I.

Effect of varying doses of ascorbic acid on (a) hypersensitivity to neoarsphenamine and (b) protection against scurvy.

Group	Ascorbic Acid mg.	Guinea Pig No.	"Flare-up" at site of 1st injection	Response to Test Dose	Gross Signs of Scurvy
I	0.25	161	(+)	(+)	+++
		163	0	0	++++
		165	+	+++	++
		168	+	++	++
		169	+	(+)	++
		170	++	†	++
		173	++	+++	++
		174	0	0	++
		177	+	++	++
		182	+	+++	++
II	0.50	106	0	0	++
		135	(+)	+	++
		141	++	+++	++
		148	++	++	++
		150	0	(+)	+
		151	0	0	+++
		157	0	0	++
III	0.75	107	0	0	0
		108	++++	+++	0
		138	++	++	0
		142	+++	+++	+
		143	+++	+++	+
		149	+	++	+
		155	+	0	0
IV	1.50	109	++	++	0
		111	++	++	0
		140	++	+++	0
		144	0	+	+
		145	++	++	0
		146	0	(+)	0
		152	+	+++	0
		154	++	++	0
		156	++	++	0
V	2.0	160	0	0	0
		164	0	(+)	0
		166	0	0	0
		167	0	0	0
		171	0	(+)	0
		172	0	0	0
		176	0	0	0
		178	+	++	0
		179	0	(+)	0
		181	0	0	0

† Died 25 days after sensitizing dose.
(+) = minimal reaction. ++++ = Maximal reaction.

guinea pigs receiving 1.5 mg. or less of ascorbic acid per day, although only those animals that received 0.5 mg. or less were definitely scorbutic. On the other hand, in Group V, fed 2.0 mg. ascorbic

acid daily, only one animal showed a pronounced reaction; and of the remaining 9, 6 showed not the slightest evidence of sensitization. The animals in this group were completely protected from scurvy. It would appear, then, that the minimum dose which seems, in our experiments, to exercise an inhibitory influence upon the sensitization to neoarsphenamine, as shown by group V, is definitely higher than that which is sufficient to protect against gross scurvy.

Those animals in the less protected groups which failed to become sensitized were frequently precisely those which exhibited the most florid symptoms of scurvy and were cachectic. It is possible that their refractoriness to sensitization was based upon a different mechanism (cachectic energy?), *i. e.,* upon a mechanism which was not operative in Group V.

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Spectographic Determination of Lead in the Blood Serum.

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The chemical methods available for the quantitative determination of lead in blood, while accurate, require amounts of blood too large for routine clinical work. Spectroscopic methods are more sensitive and allow of the use of smaller amounts of material. They have, however, been employed for qualitative estimations only.* [1, 2, 3]

We have developed a spectroscopic method for determination of lead quantitatively which using a simple procedure can estimate from 0.0005-0.01 mg. of lead with an error of ±50%.

In principle the method consists of comparing either photometrically or visually, the intensity of lead lines from the unknown sample

* Since the completion of our work an article appeared on the "Quantitative Spectographic Determination of Lead in Urine," by Jacob Cholak, *J. Am. Chem. Soc.*, 1935, **57**, 104. The technique and apparatus used, however, is more involved and expensive than that described by us.

1 Rabinowitch, I. M., Dingwall, A., Mackay, F. H., *J. Biol. Chem.*, 1933, **103**, 707; *J. Biol. Chem.*, 1933, **103**, 725.

2 Shipley, P. G., Scott, T. F. McNair, and Blumberg, H., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 327.

3 Blumberg, H., and Scott, T. F. McNair, *Bull. Johns Hopkins Hosp.*, 1935, **56**, 32.