has presented evidence indicating that pentobarbital has an atropine-like action in the cardiac vagal mechanism. If it has a similar effect on the "parasympathetic" or vagal inhibitory mechanism of the pancreas (or submaxillary), then pentobarbital would favor *prostigmine* stimulation of secretion and also *physostigmine* stimulation, which it does; and, on the basis that chloralose has the opposite action (*i. e.*, to pentobarbital) on the inhibitory mechanism, less secretion would be obtained with both *prostigmine* and *physostigmine,* which was observed. The observed effects of paraldehyde, however, do not agree with the foregoing hypothesis because paraldehyde affected the secretory response to *prostigmine* and *physostigmine* like chloralose, but not the cardiac response, although the degree of cardiac slowing was similar.

Summary and Conclusions. *Prostigmine* and *physostigmine* were administered intravenously in doses ranging from 0.005 mg to 0.2 mg per kilo to dogs anesthetized with either sodium pentobarbital, chloralose, or paraldehyde. The effects on pancreatic and salivary secretion and on the blood pressure and heart rate were recorded.

1. In the lower doses *prostigmine* is a more potent excitant of pancreatic and submaxillary secretion than *physostigmine.* A "reversal" in the response of the pancreas occurred when the dose of *prostigmine* was increased above 0.06 mg per kilo, and of the submaxillary gland in doses above 0.1 mg per kilo.

2. Chloralose anesthesia markedly diminishes the secretory response of the pancreas and submaxillary secretion to *prostigmine* and *physostigmine;* the same is true of paraldehyde anesthesia.

10680

Determination of Vitamin C Nutrition by Means of a Skin Test. A Critical Evaluation.

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(Introduced by J. H. Musser.)

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Rotter[1, 2] suggested that the state of vitamin C nutrition could be determined by means of a skin test in which 0.01 cc of a 1/400

[1] Rotter, H., *Nature,* 1937, **139**, 717.
[2] Rotter, H., *Klin. Wchnschr.,* 1938, **51**, 205.

normal solution of the dye, 2-6-dichlorphenol-indophenol, was injected intradermally and the time required for decolorization of the dye noted. He concluded that a decolorization time of more than 10 minutes indicated vitamin C deficiency; a time of 5 to 10 minutes, a normal state of nutrition; and a time of less than 5 minutes, saturation of the tissues with ascorbic acid. Portnoy and Wilkinson,[3] in a study of 25 persons, found that the mean decolorization time of the intradermal test was 16.9 minutes when the level of ascorbic acid in the blood was subnormal (0.27-0.52 mg/100 cc), 7.5 minutes when the level in the blood was normal (0.72-1.3 mg/100 cc), and 2.3 minutes when the level had been raised to 1.32 to 2.0 mg/100 cc by saturating the individual with ascorbic acid. These results indicated an inverse relationship between the time required for decolorization of 2-6-dichlorphenol-indophenol by the skin and the amount of vitamin C in the blood.

Poncher and Stubenrauch,[4] however, failed to substantiate these findings. In a study of 41 patients they found that the time of decolorization of the skin test averaged 5.8 minutes in 6 persons who had scurvy, 9.4 minutes in 9 individuals who had a subnormal level of vitamin C in the blood, and 7.6 minutes in 26 persons in whom the ascorbic acid in the blood was normal. Jetter[5] likewise found the intradermal test an unsatisfactory index of vitamin C nutrition. He studied 50 females with active tuberculosis, and in all instances the decolorization time was between 2 and 10 minutes, although the ascorbic acid in the blood varied from 0.4 to over 1.39 mg/100 cc.

This study comprises 100 observations on 45 patients, a comparison being made of the amount of ascorbic acid in the blood and the time required for decolorization of 2-6-dichlorphenol-indophenol injected into the skin. Subjects who originally showed a subnormal amount of ascorbic acid in the blood were given 200 to 300 mg of vitamin C daily and retested at intervals until the level in the blood indicated saturation of the body.

The amount of ascorbic acid in the blood was determined by the method of Farmer and Abt.[6] The skin test was performed according to the technic of Rotter, 0.01 cc of a solution of 2-6-dichlorphenol-indophenol containing 2.0 mg of the dye in 4.9 cc of distilled water being injected intradermally into the volar surface of the forearm. The dye was used either without sterilization, since it

[3] Portnoy, B., and Wilkinson, J. F., *British M. J.*, 1938, **1**, 328.

[4] Poncher, H. G., and Stubenrauch, C. H., *J. A. M. A.*, 1938, **111**, 302.

[5] Jetter, W. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 169.

[6] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 146.

TABLE I.

No. tests	Ascorbic acid Blood plasma (mg%)	Decolorization time-intradermal test (min) Range	Mean	Standard deviation
26	0.00-0.69 (Subnormal)	2.0-14.75	8.69 ± 0.383	2.89 ± 0.271
42	0.70-1.29 (Normal)	2.0-17.50	6.93 ± 0.322	3.10 ± 0.228
32	1.30-1.99 (Saturated)	3.0-15.40	8.31 ± 0.321	2.69 ± 0.227

was found to be bacteria-free, or after passage through a Seitz filter, as suggested by Portnoy and Wilkinson.[8] This latter manipulation did not affect the results. Since part of the discrepancy in the findings of various workers could have been due to the amount of dye injected intradermally, a device[7] was employed which allowed delivery of exactly 0.01 cc from a tuberculin syringe. To avoid another possible cause of inconstant findings, that is, differences in blood flow due to the position of the extremity, the patient was always seated with the forearm resting slightly below the heart level on a table.

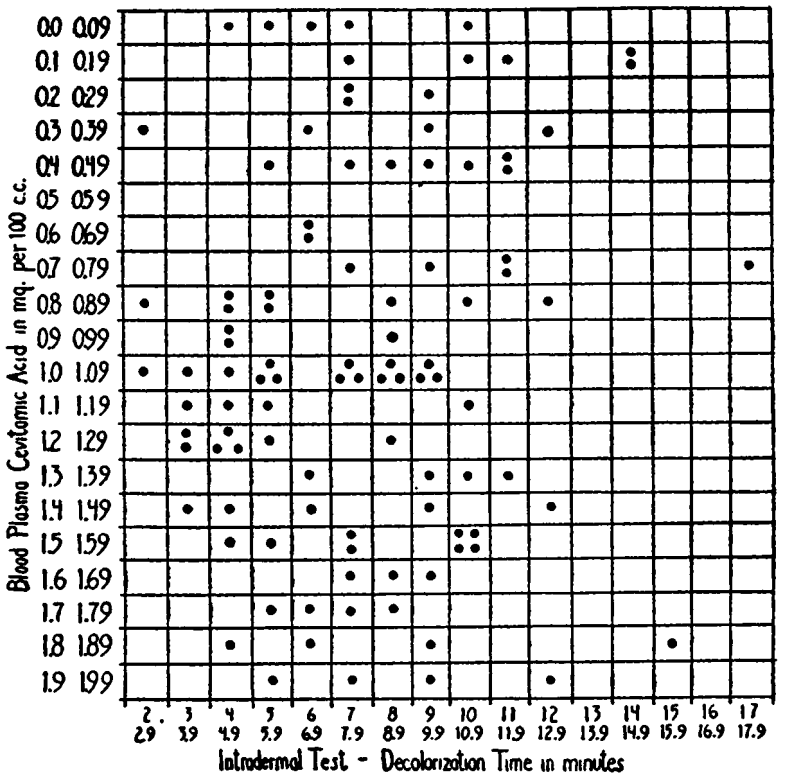
Table I shows the relation between the time of decolorization of the intradermal tests with 2-6-dichlorphenol-indophenol and the level of ascorbic acid in the blood plasma in 100 tests. It is evident that the time required for fading of the dye shows the same variation whether the amount of vitamin C in the blood was high or low. If the mean decolorization time of skin tests in the group with a normal quantity of ascorbic acid in the blood is compared with that of the group showing subnutrition, there is a difference of 2.76 ± 0.50, which is statistically significant. However, if the mean decolorization time of the skin tests of the persons who had subnutrition is compared with that of the persons who were saturated, the difference is 0.379 ± 0.499, obviously not statistically significant.

The complete lack of correlation between the decolorization time of the skin test with 2-6-dichlorphenol-indophenol and the content of ascorbic acid in the blood is shown in Fig. 1. The coefficient of correlation for the 100 tests was found to be —0.106 ± 0.067. It is obvious that the skin test in its present form cannot be used as a substitute for examination of the blood in determining the state of vitamin C nutrition.

Examination of the data obtained in individuals who had repeated skin and blood tests at different levels of vitamin C nutrition (subnormal, normal and saturated) suggested that there might be some correlation in a given individual between these two determinations. Accordingly, for each of 9 patients who had had more than 5 tests, the percentage deviation of each determination from the mean for

[7] Burch, G. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 676.

FIGURE ONE



that individual was calculated. The correlation coefficient was again not significant, being 0.293 ± 0.092.

In order to estimate the magnitude of the error of a single intradermal test, one individual was given a series of 5 injections in the forearm every 15 minutes until a total of 25 injections was reached. The time of decolorization of the dye varied from 4.5 to 11.6 minutes with a mean of 8.9 ± 0.283 minutes. The standard deviation was 2.097 ± 0.199. These figures correspond closely with those obtained in the study of the patients who showed vitamin C subnutrition or saturation.

There are a number of factors which might affect the variability of an intradermal test, especially when this test depends upon the quantity of reducing substances in the skin. Vitamin C is only one, and possibly not the most important, reducing agent present. Changes in the circulation and in the amount of oxygen available in the tissues might influence the rate of reduction of the dye. Variations in room temperature and in the position of the forearm could

be important in this regard. The lymphatic drainage of the forearm might also be a factor in the rate of decolorization.

Several procedures were used to determine the effect of circulatory changes and anoxemia on the intradermal test. Arterial occlusion was produced by a blood pressure cuff for 5 to 15 minutes in one group of experiments. In another, pressure was applied to an area of the forearm for 5 minutes by a strip of X-ray film to which 2,000 gm of weight was attached. Both procedures resulted in a more rapid disappearance of the dye than in control tests. In the second experiment, the speed of decolorization may have been due in part to pressure which forced the dye into the deeper lymphatics rather than to ischemia of the underlying tissues. Changing the room temperature from 26°C to 18°C increased the time required for disappearance of the dye in one individual.

At times when a series of intradermal tests was performed in the same individual, one wheal would fade suddenly, while the remainder would disappear slowly over a long period of time. This phenomenon may be due to a sudden rupture of some of the tissue spaces.

Administration of 1,000 mg of vitamin C intravenously caused a rise in the ascorbic acid in the plasma from 0.15 to 1.40 mg/100 cc to a level of 6.0 to 8.0 mg/100 cc. During this period the decolorization time of the dye was reduced to about one-half of the original figure. Changes of this magnitude in the amount of vitamin C in the blood and tissues do not occur under ordinary circumstances.

Conclusion. In 100 tests there was no correlation between the amount of ascorbic acid in the blood during fasting and the time of decolorization of an intradermal injection of 2-6-dichlorphenol-indophenol. This skin test is not a satisfactory method for the determination of the state of vitamin C nutrition.