TABLE 1

REDUCED AND DEHYDROASCORBIC ACID IN CANTALOUPE BEFORE AND AFTER MACERATION*

	Ascorbic acid mg/100 gm		
	Reduced	Total	Per cent. dehydro
Before maceration	49	51	4
After maceration	0	49	100
After maceration and holding 4 hours	0	41	100

* 1 minute in Waring Blendor.

tract, following the method of Bessey,[2] it was found that almost all the ascorbic acid was still present but was in the reversibly oxidized form. The conversion was evidently catalyzed by the presence of ascorbic acid oxidases, for when the cantaloupes were steamed before they were macerated, the ascorbic acid remained in the reduced form.

The dehydroascorbic acid which had been so quickly formed when the melon cells were ruptured was evidently itself much more slowly changed to a biologically inactive substance. After holding the raw macerated material in which all the ascorbic acid was in the dehydro form for 4 and 24 hours, the loss in total ascorbic acid was only 16 and 35 per cent., respectively.

The possibility that conversion of reduced to dehydroascorbic acid may occur in raw vegetables when prepared for consumption, as quickly as it did in melons when macerated, suggested itself. As recently reported in SCIENCE,[3] "In many mess halls vegetables and fruits are finely minced." The losses in ascorbic acid which resulted in 30 minutes and 2 hours after mincing with steel or plastic knives or in the "Buffalo" chopper were reported. Although it was stated that "analyses were run by the method of Bessey,"[2] whether total ascorbic acid or the reduced form only was determined is not clear.

From our data, as shown in Table 2, it appeared that when apples, celery, cucumbers, peppers and radishes were chopped as is commonly done when they are served in salads, or when the juice was extracted as in Health Food Stores, a high percentage of the reduced ascorbic acid which had disappeared was still present in a biologically active form. Although some of the dehydroascorbic acid was destroyed on standing 4 or 24 hours, the losses were relatively small. When potatoes were boiled a loss in reduced ascorbic acid occurred as expected, but the action of ascorbic acid oxidase which catalyzes the conversion of the reduced to the dehydro form had obviously been inhibited. When ripe tomatoes were sliced, or their juices expressed, then ascorbic acid remained in the reduced form. The opposite was true for *green* tomatoes, for their ascorbic acid when juiced was all converted to the reversibly oxidized form and remained in this form after holding 4 hours.

TABLE 2

EFFECT OF PREPARATION OF RAW FOODS FOR CONSUMPTION ON THEIR VITAMIN C VALUES

Food	Method of preparation before sampling	Ascorbic acid/100 gm			
		Reduced		Total (Reduced and dehydro)	
		mg	Per cent. loss	mg	Per cent. loss
Apples	Uncut	3	...	5	...
	Chopped	0	100	5	0
	Juice	0	100	5	0
Celery	Uncut	8	...	12	...
	Chopped	5	38	12	0
	Juice	0	100	9	25
Cucumbers	Uncut	9	...	13	...
	Chopped	5	44	12	9
Peppers (green)	Uncut	70	...	77	...
	Chopped	38	46	74	4
	Juice	0	100	60	22
Potatoes	Uncut	30	...	32	...
	Chopped	3	90	28	13
	Boiled	21	30	24	25
	Boiled and mashed	10	67	14	55
Radishes	Uncut	28	...	31	...
	Chopped	19	32	29	10
Tomatoes (red)	Uncut	16	...	19	...
	Sliced	16	0	19	0
	Juice	16	0	19	0
(green)	Uncut	16	...	20	...
	Juice	0	100	20	0

Evidently the extent and rapidity of the conversion varied greatly with a food, its pH, enzymes present and the method of preparation for consumption.

The data in this paper thus indicate that the conversion of reduced ascorbic acid to the reversibly oxidized form proceeds so rapidly in some foods under many different conditions of sampling, holding, and preparation for consumption, and is frequently of such magnitude, that the usual method of assay of the reduced form *only* is an inadequate measure of the true vitamin C values if dehydroascorbic acid may be considered only slightly less active as an antiscorbutic than the reduced form.

MARGARET CAMMACK SMITH
EMILY CALDWELL

DEPARTMENT OF HUMAN NUTRITION,
ARIZONA AGRICULTURAL EXPERIMENT STATION
TUCSON

THE EXPERIMENTAL TRANSMISSION OF PINTA, MAL DEL PINTO OR CARATE TO THE RABBIT[1]

MOOSER, Varela and Vargas,[2] León-Blanco,[3] Briceño Rossi and Iriarte[4] and Lieberthal[5] tried, unsuccess-

[2] O. A. Bessey, *Jour. Biol. Chem.*, 126: 771–784, 1938.

[3] C. M. McCay, M. Pijoan and H. R. Taubken, SCIENCE, 99: 454–5, 1944.

[1] From the Instituto de Enfermedades Tropicales y Parasitología "Carlos Finlay," University of Habana:

fully, to infect laboratory animals with *Treponema carateum,* the causal agent of pinta, mal del pinto or carate.

Curbelo and collaborators[6] were able to produce keratitis and orchitis, respectively, in rabbits, utilizing as inoculum material obtained from a Cuban case of pinta, but the lesions by them described were not wholly characteristic, and could have been produced by banal microorganisms or by *Treponema pallidum.* The latter possibility must be the more seriously considered, since León-Blanco demonstrated that the cutaneous syndrome known as pinta in Cuba may be caused by both *Treponema carateum*[7] and *Treponema pallidum.*[8]

In this preliminary note we report the results obtained at the beginning of a series of experimental studies started with the object of differentiating between the pinta-like cutaneous syndrome produced by *T. pallidum* (and which constitutes a still undetermined proportion of what in Cuba is known as "pinta") and true pinta, mal del pinto or carate, a well-defined nosologic entity produced by a specific treponeme: *Treponema carateum,* Brumpt 1939 or *Treponema herrejoni,* León-Blanco 1939.

Material and methods: One of us (A. O.) saw in December, 1943, the patient L. M., a Jamaican negress, 47 years old, who presented a keratotic, dyschromic skin condition affecting the palms and soles, accompanied by intensely positive Kahn and Meinicke reactions, and diagnosed clinically as pinta, mal del pinto or carate. We could easily demonstrate treponemata in the skin lesions by means of the technique recommended by one of us.[3]

In order to prove conclusively the cause of this patient's illness, we inoculated on January 4, 1944, a volunteer (A. A.), in the anterior aspect of the left forearm, with serous fluid expressed from the dyschromic lesions, utilizing the technique one of us (F. L. B.) has introduced for the experimental transmission of pinta, mal del pinto or carate from man to man. At each of the two points of inoculation there appeared the initial lesions of pinta, and in these the presence of treponemata could readily be demonstrated.

The initial lesions have continued to progress, and at the time of writing the first efflorescence of pintids has appeared, *i.e.,* the first lesions of the stage of general spread of the disease. This experimental proof shows beyond the shadow of a doubt that our patient, L. M., had pinta, mal del pinto or carate, and not syphilis.

On February 18, 1944, we obtained a few drops of serous fluid by compression of the initial lesions of A. A., and dark-field examination showed the presence in the fluid of *Treponema carateum.* The fluid, diluted with 0.4 cc of normal salt solution, constituted the inoculum. With a syringe and needle of the kind used for the Mantoux tuberculin test we inoculated four black rabbits intradermally in the scrotum, as superficially as possible, with 0.1 cc for each animal.

Results: The rabbits were examined at weekly periods. June 13, 1944, 105 days after inoculation, there was visible in one of the rabbits, at the very point of inoculation, a pink papule that grew slowly to a diameter of 8 mm. June 23, 1944, 115 days after inoculation, the summit of the papule presented a circular superficial erosion 5 mm across; the erosion was covered by a dark scab. Upon lifting the scab several droplets of bloody serum oozed from the base of the erosion, and in this fluid we found numerous treponemata by dark-field examination; they stained easily with the Fontana-Tribondeau method.

Four additional rabbits were inoculated on June 24, 1944, with the serous fluid from the eroded papule of the above rabbit, but to this date no lesions have appeared. In order to identify the treponemata observed in the eroded scrotal papule of the rabbit we inoculated that same day (June 24, 1944) a second volunteer, D. D., in the skin of the volar aspect of the left forearm. A traumatic inflammatory reaction developed at the point of inoculation, but this disappeared in 72 hours. July 7, 1944, 13 days after inoculation, a rounded zone of erythema, 2 mm across, was noticed at the point of inoculation. July 17 the zone of erythema was slightly infiltrated and somewhat papular. It grew slowly, and by August 17, 1944, had the appearance of an ovoid papule measuring 6 by 4 mm in principal diameters; the surface was finely scaly. On August 10, 1944, we expressed a few drops of serous fluid from the papule and demonstrated by dark-field examination numerous treponemata that were easily impregnated with the Fontana-Tribondeau method. Because of the manipulations necessary to obtain the serous fluid, D. D.'s initial lesion at present shows slight ulceration that is undergoing healing. The rabbit's eroded papule began to heal, and disappeared completely on July 12, 1944, at which time the

Director: P. Kourí. Abstracted from a preliminary note read before the Cuban Society of Biology, Tropical Medicine and Parasitology on August 17, 1944.

[2] H. Mooser, G. Varela and L. Vargas, *Bol. inst. hig.,* 2: 224, January, 1936.

[3] F. León-Blanco, *Rev. med. trop. y parasit., bact., clin. y lab.,* 6: 5, January–February, 1940.

[4] A. L. Briceño Rossi and D. R. Iriarte, *Bol. lab. clin. l. razetti,* 4: 221, March, 1944.

[5] E. P. Lieberthal, *Jour. Am. Med. Asn.,* 123: 619, November, 1943.

[6] A. Curbelo *et al., Rev. cienc. med.,* 1: 134, October, 1938.

[7] F. León-Blanco, *Arch. soc. est. clin. hab.,* 35: 165, March, 1941.

[8] F. León-Blanco and L. Beausoleil, *Arch. soc. est. clin. hab.,* 38: 257, January–February, 1944.

scab dropped off spontaneously. The papule has been converted into a nodular infiltration the size of a grain of corn, which we are continuing to observe closely.

Comments: Patient L. M. undoubtedly had pinta, mal del pinto or carate, as shown by the inoculation of volunteer A. A., in whom the initial lesions of pinta, mal del pinto or carate were produced at each one of two points of inoculation. In the present state of our knowledge the only laboratory method that will allow with certainty to differentiate syphilis from pinta, mal del pinto or carate is the experimental inoculation of man, with the production of an initial pinta lesion different from that of syphilis and pian.

The ulcerative papular lesion produced in one of four rabbits inoculated with serous fluid obtained from the initial pinta lesion of volunteer A. A. resulted from the inoculation of *Treponema carateum* and not from some other species of treponema, since subpassage to volunteer D. D., utilizing serous fluid expressed from the ulcerated papule of the rabbits, resulted in the development of an initial pinta lesion that contained abundant treponemata.

Summary: In this preliminary note we describe the results obtained by inoculation of the skin of the scrotum of four black rabbits with serous fluid expressed from an initial pinta lesion. The inoculation was made intradermally, as superficially as possible, with serous fluid rich in treponemata and diluted with normal salt solution. A papule appeared at the point of inoculation in one of the four rabbits on the 105th day. One hundred and fifteen days after inoculation the papule presented a circular erosion on an indurated base. The serous fluid expressed from this lesion contained numerous treponemata on dark-field examination, and these were easily impregnated by the Fontana-Tribondeau method. A volunteer inoculated with serous fluid expressed from the rabbit's lesion developed a typical initial pinta lesion at the point of inoculation, thus proving that the scrotal lesion of the rabbit was produced by *Treponema carateum* or *Treponema herrejoni,* the causal agent of pinta, mal del pinto or carate.

Four rabbits inoculated with serous fluid from the scrotal lesion of the rabbit have not yet developed visible lesions.

FRANCISCO LEÓN-BLANCO
ALBERTO OTEIZA

HABANA, CUBA

THE PROTECTION OF PANCREATIC LIPASE BY ACACIA GUM

DURING a series of experiments on the enzymatic activity of pancreatic lipase, it was found that the presence of the acacia gum had a pronounced effect in preventing the destruction of the enzyme. An emulsion consisting of 1 ml tributyrin plus 1 gm of acacia gum plus 100 ml of water buffered to a pH of 8.8 was the substrate used in these experiments. The substrate was added to 80 mg of fat-free pancreatic lipase. The reaction was allowed to proceed at constant temperature in a water bath. The mixture was agitated constantly by means of an electric stirrer. To measure the pH a glass and a calomel electrode were placed in the mixture and connected to an electronic null indicator and potentiometer. The mixture was maintained at constant pH by adding sodium hydroxide at short

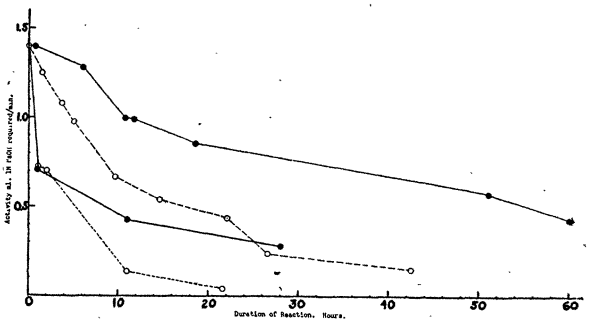

FIG. 1. Residual lipase activity after pancreatic lipase was in solution with various substrates for different periods of time. Upper Curves: Enzyme with tributyrin with gum ————. Enzyme with tributyrin without gum - - - - - - - -. Lower Curves: Enzyme with gum without tributyrin ————. Enzyme in buffer without gum and without tributyrin - - - - - - - -.

intervals of time. The reactions were allowed to proceed for varying durations from forty minutes to sixty hours. To determine the amount of enzyme activity remaining after each period an emulsion containing 10 ml of tributyrin was added and the rate of the reaction followed for at least fifteen minutes. The velocity of this hydrolysis was taken as a measure of the residual enzyme activity.

The following controls were also run: substrates of tributyrin with no acacia gum, gum plus enzyme with no tributyrin, and enzyme without gum and without tributyrin. Fig. 1 shows the results of these experiments. It may be seen that the enzyme deteriorates most rapidly in the absence of gum and tributyrin and that the presence of gum without tributyrin protects the enzyme to a large extent. In the emulsions containing tributyrin the presence of gum further protects the enzyme from destruction. One experiment in which 40 gms of gum were used instead of 1 gm a rate of 1.3 ml of 1N NaOH per minute after 615 minutes of enzyme action was obtained. This represents only a slight enzyme deterioration.

The mechanism by which the gum protects the enzyme is at present unknown, but Mendel and Rudney[1] have recently discovered that acacia gum and

[1] B. Mendel and H. Rudney, SCIENCE, 100: 499, 1943.