pigeon breast muscle to which insulin had been added (as compared to a control maintained under the same conditions for a similar period of time) is accompanied by an increased ability to utilize pyruvic acid (Table I).

We have found, further, that this pyruvate utilization can be inhibited by malonate and restored, as Krebs and Eggleston have demonstrated in the case of fresh suspensions of pigeon muscle,[7] by the addition of fumarate + pyruvate and of pyruvate + oxaloacetate. While both of these reactions occur at a greater rate in the insulin-supplemented tissue, the rates of citrate and α-ketoglutarate oxidation are unaffected by the presence of the hormone (Table II).

These data demonstrate for the first time a direct *in vitro* association between the action of insulin and the oxidation of a carbohydrate substrate, namely, pyruvic acid. They suggest further that insulin is concerned in maintaining the functional integrity of either one or both of the enzyme systems involved in the reactions of fumaric and pyruvic acid or of oxaloacetic and pyruvic acid.

These experiments will be reported in greater detail elsewhere.

LESTER RICE
E. A. EVANS, JR.

DEPARTMENT OF BIOCHEMISTRY,
UNIVERSITY OF CHICAGO

SCIENTIFIC APPARATUS AND LABORATORY METHODS

ISOLATION OF AN ACTIVE SUBSTANCE FROM CALONYCTION ACULEATUM CAPABLE OF COAGULATING CASTILLA LATEX

CASTILLA latex is different from Hevea latex in that it is not easily coagulated by common chemical reagents. For many years, a juice prepared by natives of Central America from the moonvine of Nacta vine (*Calonyction aculeatum* formerly *Ipomea bona-nox*) has been used to coagulate the latex tapped from the Castilla tree. The origin of this discovery is apparently unknown. With increased interest in Castilla rubber resulting from the present rubber emergency, it has been necessary to seek some method for coagulating Castilla latex on a commercial scale. Trafton[1] has devised a method by which the latex is creamed, washed and finally coagulated with chemicals, but the method requires that the pH of the latex be rather rigidly controlled during processing, a condition not always attainable under field conditions, particularly when native labor is used. The native method of coagulation with Nacta extract would continue to be reasonably satisfactory except for two problems: (1) The vine has been almost completely exterminated in its former habitats, where it was associated with Castilla trees, and (2) there are areas in which moonvine has never been found in association with Castilla. Hence, the desirability of isolating the active principle from Nacta vine has been suggested as offering a method whereby a dried extract or some other suitable concentrate might be prepared in one area to be shipped to some other area where Castilla latex is to be coagulated. As the following directions will indicate, this laboratory has been successful in isolating from Nacta a material which is very active in coagulating Castilla latex under laboratory conditions.

METHODS AND MATERIALS

Calonyction aculeatum grows abundantly in southern Florida. It has been possible, therefore, to have ample material shipped in from this source so as to arrive in optimum condition (material shipped from Mexico decayed in transit). Preliminary experiments indicated that no loss in activity was experienced when the vine was rapidly dried *in vacuo* at 70° C; similarly, it was found that the substance responsible for coagulating Castilla latex was not soluble in water, but was readily soluble in ethyl alcohol, acetone, ethyl ether, petroleum ether and benzene. With these facts in mind, the following procedure was adopted in preparing an active material.

Ten grams of dry stems of *C. aculeatum,* ground to pass 40 mesh, were extracted with ethyl ether for 12 hours in a Soxhlet apparatus. At the expiration of this period, the green ether extract was transferred to an evaporating dish and the ether removed, leaving a sticky mass of material heavily charged with chlorophyll. This was then dissolved in a small quantity of benzene, transferred to a beaker and activated charcoal added. The material was heated on a steam bath for about ten minutes to insure adequate adsorption. Filtration of the benzene extract to which charcoal had been added disclosed a yellow-colored filtrate from which most, if not all, of the photosynthetic pigments had been removed by adsorption on carbon. The filtrate was evaporated to dryness leaving a resinous mass of yellow color. This material was dissolved in a small quantity of acetone and then dispersed into approximately 30 ml of water, producing a white, cloudy, colloidal sol which, when viewed by reflected light, appeared to have a reddish tinge. The acetone was removed from the sol by warming on a steam

[7] H. A. Krebs and L. V. Eggleston, *Biochem. Jour.*, 34: 442, 1940.

[1] Unpublished data.

bath until the odor of acetone could no longer be detected. The hydrosol was cooled in an ice bath to about 5° C. Upon standing overnight in an ice chest, a yellow substance separated from the sol. When subsequently centrifuged at 4,500 RPM for 15 minutes, all the material was precipitated, leaving a clear supernatant liquid. The precipitated material was washed several times with water, the water decanted, the precipitate redissolved in acetone, filtered, redispersed into water, and the acetone removed as before. Centrifuging caused a clear, yellow, resin-like substance to collect at the bottom of the centrifuge tube. The resin was gathered on a stirring rod and removed from the tube for drying. From ten grams of dry plant material, 400 milligrams of dried resin were obtained, or a yield of about 4 per cent. on a dry weight basis.

Coagulation Tests of Calonyction Resin with Castilla Latex

The Castilla latex used in the following experiments was received from Mexico and labeled "Latex Castilla por de Pichucalco," and was collected on September 15, 1942. The shipment arrived in Washington on October 14, 1942, in apparently good condition. Ten milliliters of undiluted latex were measured in a graduate, poured into a 30 ml beaker and the desired amount of sol added for coagulative tests. The beakers were kept covered to diminish surface oxidation of the latex during the time of the test. When coagulation occurred, the rubber was separated from the serum at the end of 14 hours, washed several times in water, and weighed after being superficially dried in a low temperature oven. In some cases, the serum also was evaporated to dryness for rubber content determination. Resin and rubber analyses were made by the Bailey-Walker method using acetone and benzene as solvents for resins and rubber, respectively.

A sol containing 0.82 mg of resin per milliliter of water, and another sol containing 0.47 mg of resin per milliliter were prepared by first dissolving the resin in a small amount of acetone, dispersing the resin into water with stirring, removing all the acetone by warming, and then making the sol to a known volume with water. Tables I and II illustrate the coagulating powers of these sols, and the composition of the coagulum and serum.

TABLE I

Coagulative Power of a Calonyction Resin Sol Containing 0.47 mg of Resin Per Milliliter of Water. Ten Milliliters of Latex Used in All Tests

Ml of sol	Mg of nacta resin	Time of coagulation	Weight of rubber in grams
1.0	0.47	None in 12 hours	0
2.0	0.94	None in 12 hours	0
4.0	1.88	Begins in 30 minutes	2.35
8.0	3.75	Begins in 10 minutes	2.95
16.0	7.52	Begins almost at once	2.68

TABLE II

Coagulative Power of a Calonyction Resin Sol Containing 0.82 mg of Resin Per Milliliter of Water. Ten Milliliters of Latex Used in All Tests

Ml of sol	Mg of nacta resin	Time of coagulation	Per cent. rubber coagulated	Coagulum			Serum	
				Weight in grams	Per cent. resins	Per cent. rubber	Weight in grams	Per cent. rubber
0.5	0.41	None in 14 hours	0	0	...	....	10.32	...
1.0	0.82	None in 14 hours	0	0	...	....	10.32	...
2.0	1.64	10 minutes ±	20.7	2.14	5.60	72.34	8.18	5.63
4.0	3.28	5 minutes ±	23.3	2.41	6.37	58.27*	7.91	1.78
8.0	6.56	2 minutes ±	23.4	2.42	7.07	76.60	7.90	0.78

* Benzene extraction of rubber not complete after 32 hours.

These data are suggestive of the use that this resin may find in the commercial production of Castilla rubber. Since, however, absolutely fresh latex has been unavailable, we are hesitant in predicting the coagulative powers of Nacta resin under field conditions, and for this reason, we are withholding comment and interpretation of the data contained in the tables until the results of further trials on fresh latices have been ascertained.

S. G. Wildman
A. V. McMullan
Rosamond Griggs

Rubber Plant Investigations,
Bureau of Plant Industry,
U. S. Department of Agriculture

BOOKS RECEIVED

Gemmill, Chalmers L. *Physiology in Aviation.* Illustrated. Pp. vii + 129. Charles C Thomas. $2.00.

Hyslop, J. M. *Infinite Series.* Illustrated. Pp. xi + 120. Interscience Publishers. $1.75.

Knapp, E. J. *Basic Physics for Pilots and Flight Crews.* Illustrated. Pp. v + 118. Prentice-Hall. $1.65.

McCrea, W. H. *Analytical Geometry of Three Dimensions.* Illustrated. Pp. vii + 144. Interscience Publishers. $1.75.

Masserman, Jules H. *Behavior and Neurosis.* Illustrated. Pp. xv + 269. The University of Chicago. $3.00.

Wilks, S. S. *Mathematical Statistics.* Illustrated. Pp. 284. Princeton University Press. $3.75.

Winning, J. *Heat Treatment of Metals.* Illustrated. Pp. 99. Chemical Publishing Company, Inc. $1.50.