O-butylation renders the quaternary base practically inactive.

In conclusion, we wish to point out that the availability of pure crystalline preparations with high curare activity will fill an urgent need for well-defined material for physiological and clinical experimentation.

SUMMARY

Crystalline d-tubocurarine has been isolated in good yield from curare prepared from a single plant species, namely, *Chondodendron tomentosum*. This result establishes with certainty the botanical origin of this compound and substantiates the supposition that it is this species which furnishes the active constituent in certain types of curare.

The extract from this plant furthermore yielded two new tertiary alkaloids which could be converted into physiologically active quaternary bases.

Methylation of the phenolic hydroxyl groups in the quaternary bases resulted in a 3–9-fold increase in physiological potency.

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THE IN VITRO EFFECT OF INSULIN IN PIGEON BREAST MUSCLE[1,2]

IN 1938 Krebs and Eggleston[3] demonstrated an *in vitro* oxidative effect of insulin on a suspension of minced pigeon breast muscle in phosphate buffer to which has been added certain oxidizable substances. The effect was especially pronounced in the presence of citric acid. While these observations have been confirmed by other investigators,[4,5,6] the site of action of insulin in this experimental system is unknown, although Krebs and Eggleston concluded that their evidence "suggests that insulin acts as a catalyst in the citric acid cycle."

It seemed possible to us that information in regard to the action of insulin in this experimental system could be obtained by studying the respiration of a suspension of minced pigeon breast muscle during the period when the insulin effect is present.

TABLE I

THE EFFECT OF INSULIN ON AEROBIC PYRUVATE REMOVAL

Two flasks contained 2.5 gm of minced pigeon breast muscle in 22.5 ml of calcium-free phosphate saline (pH 7.4) + 5.0 ml boiled muscle extract. One flask (enzyme A) received 1.5 ml phosphate buffer; the other (enzyme B), 1.5 mgm zinc-free insulin in 1.5 ml phosphate buffer. Both vessels were gassed with 100 per cent. O_2. 4.0 ml samples from each flask were placed in Warburg vessels, gassed with 100 per cent. O_2 and shaken at 40° C. until these pilot vessels showed the beginning of the insulin effect (ca. 80 minutes). The reserve flasks which had been shaken at 40° during this time were removed from the water bath and 4 ml of the enzyme suspensions + other additions were added to Warburg vessels as indicated in the table. The vessels were gassed with 100 per cent. O_2, equilibrated at 40° C. for 10 minutes, and substrates tipped in from the side arm. 20 per cent. KOH was placed in the center cup. Total volume of liquid: 4.7 ml. Experimental period, 25 minutes. Pyruvic acid was measured by the carboxylase method.

Experiment:	1		2		3	
Vessel:	1	2	1	2	1	2
Enzyme A (ml.)	4.0	....	4.0	....	4.0	....
Enzyme B (ml.)*	....	4.0	....	4.0	....	4.0
Pyruvate added (μl.)	431	431	373	373	393	393
Pyruvate utilized (μl.)	91.5	234.0	69	191	224	300
O_2 uptake (μl.)	390	475	441	475	634	944

* 1.1 units of insulin per ml.

In experiments to this end, we have found, first, that the greater oxygen uptake of a suspension of

TABLE II

EFFECT OF INSULIN ON THE O_2 UPTAKE AND PYRUVATE REMOVAL IN MALONATE-POISONED SYSTEMS

All manipulations are the same as those recorded in Table I. Malonate added to the vessel directly. The vessels were run for 70 minutes. The data in this table are from the same tissue suspension used in Experiment 2, Table I.

	Vessel									
	1	2	3	4	5	6	7	8	9	10
Enzyme A added (ml)	4.0	.....	4.0	.....	4.0	.....	4.0	.....	4.0	.....
Enzyme B added (ml)*	.....	4.0	.....	4.0	.....	4.0	.....	4.0	.....	4.0
Malonate conc. (M)	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019
Fumarate added (μl)	.....	.....	224	224	.....	.....	.....	.....	.....	.....
Oxaloacetate added (μl)	.....	.....	.....	.....	224	224	.....	.....	.....	.....
Citrate added (μl)	.....	.....	.....	.....	.....	.....	448	448	.....	.....
α-Ketoglutarate added (μl)	.....	.....	.....	.....	.....	.....	.....	.....	448	448
Pyruvate added (μl)	373	373	373	373	373	373	.....	.....	.....	.....
Pyruvate recovered (μl)	275	246	235	167	360	311	.....	.....	.....	.....
Pyruvate utilized (μl)	98	127	138	206	†	†	.....	.....	.....	.....
O_2 uptake (μl)	159	194	272	452	222	282	183	192	164	154

* 1.1 units of insulin per ml.

† Calculation of pyruvate utilization in the presence of oxaloacetate is impossible since oxaloacetate yields 2 mols CO_2 in the carboxylase method and is also decarboxylated to an unknown degree when added to tissues. These data, however, indicate an increased pyruvate uptake in the presence of insulin and oxaloacetate.

[1] This investigation was supported in part by a grant from Armour and Company.

[2] The work reported here was done by Lester Rice in partial fulfilment of requirements for a Ph.D. in biochemistry, Division of Biological Sciences, University of Chicago.

[3] H. A. Krebs and L. V. Eggleston, *Biochem. Jour.*, 32: 913, 1938.

[4] E. Shorr and S. B. Barker, *Biochem. Jour.*, 33: 1798, 1939.

[5] F. J. Stare and C. A. Baumann, *Cold Spring Harbor Symposia on Quantitative Biology*, 7: 1939.

[6] W. C. Stadie, John A. Zapp, Jr., and F. D. W. Lukens, *Jour. Biol. Chem.*, 132: 411, 1940.

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.

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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.