two hundred years since they were last published in Latin. Through the devoted labor of Professor Adelmann these books, the foundation stones of modern embryology, are now set before us in a noble volume which contains the two Latin texts in facsimile, with English translations which are both readable and scholarly, entertaining biographical notes and instructive commentaries, copious annotations and cross-references and a detailed bibliography.

The studies of Fabricius were of course made without the microscope. They concern the structure of the reproductive organs of the hen, the structure of the egg and the way in which the embryo is laid down. The observations on the mammalian fetus concern almost exclusively the placenta, membranes and fetal blood vessels. As Professor Adelmann points out, the ultimate goal of Fabricius, as of Aristotle and Galen, was "to explain causes, and particularly to elucidate the final cause, the end or purpose served by each part." Structure and function were studied primarily for their aid in the comprehension of the end or useful purpose. These two books therefore are couched in a tone of scholastic inquiry which requires (and receives) a good deal of explanation by the translator in order to make them clear to the present-day reader. In his introductory chapters, Adelmann traces the previous history of embryology from Aristotle through Galen and the sixteenth century writers, including Vesalius and Coiter. Then, in a careful analysis of the text of Fabricius, he shows us how the latter began his work saturated with the spirit and point of view of Aristotle and Galen and how he had to adjust his observations of fact to the doctrinal patterns of his times.

There has been a tendency to over-emphasize the traditionalism and the factual errors of Fabricius. He made several striking mistakes, such as deriving the chick from the chalazae of the egg; but these are completely outweighed by a host of careful and (for the time) accurate descriptions of the egg and the chick, of the mammalian placenta and membranes and of the umbilical and fetal vessels. He studied a very wide range of species, and was the first to describe and illustrate in print the diffuse placenta of the pig and horse and the human decidua. The illustrations which accompany his texts are remarkably clear and instructive, and many of them could still be used for teaching. They are well reproduced in this volume.

The reviewer has perhaps said enough to indicate that Dr. Adelmann has provided much more than reprints and translations of these books. He has shown us their proper place in the history of embryology and has made it possible for students in our day to understand the achievement of their author.

Students of Harvey will find here a careful study of the relations between his work and that of Fabricius. In the translations, all the more important passages which Harvey quoted from Fabricius are specially indicated.

This work, from its touching Latin dedication to the memory of Dr. Adelmann's mother and sister, through to its excellent index, is a monument of scholarship—learned, thorough and withal interesting, and satisfyingly complete. Students of embryology and of the history of science, now and in the future, will be grateful not only to the author, but also to Cornell University, the Council of Learned Societies and the Carnegie Corporation, for making its publication possible. Special mention should be made of the handsome format, and of the typography designed by Robert Josephy, which combines beauty and legibility with a clever suggestion of seventeenth century style, making the English translations and the commentaries appear fully compatible with the dignified Paduan printing of the Latin texts.

GEORGE W. CORNER

SPECIAL ARTICLES

CURARE ALKALOIDS FROM CHONDODENDRON TOMENTOSUM

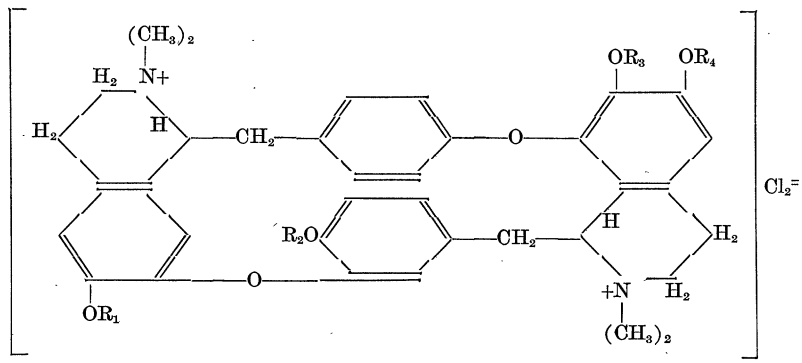
CURARE is the generic name for a group of highly effective arrow poisons of plant origin used by the South American Indians. Recent clinical work has given encouraging indications that this drug, with its powerful lissive action on the voluntary musculature, might become a valuable therapeutic agent in the treatment of spastic paralysis, for moderating the convulsions in the shock-therapy of certain psychoses, and as an adjunct to anesthesia in surgery. So far, the chief obstacle to the therapeutic use of curare has been the widely varying potency and the uncertain origin and composition of the available preparations.

The isolation of a physiologically active, crystalline alkaloid from curare proved to be a difficult task. After numerous unsuccessful attempts by other workers, H. King, in 1935, finally announced the isolation of a crystalline, highly active quaternary base chloride, designated by him d-tubocurarine chloride, from a specimen of tube curare.[1] The earlier work of M. Scholtz, of E. Spaeth and of F. Faltis on the inactive tertiary base, l-curine from curare, and the related alkaloids bebeerine and isobebeerine (isochondodendrine) found in the drug *pareira brava,* enabled King to establish the structure of d-tubocurarine chloride as that of a bisbenzylisoquinoline alkaloid in which the nitrogen atoms are quaternary (formula I). On the

[1] H. King, *Jour. Chem. Soc.* (London) 1381, 1935.

basis of a subsequent chemical investigation of pot curare[2] and of the tertiary alkaloids from various menispermaceous plants[3] this author expressed the belief that the still unsettled problem of the botanical provenance of tube- and pot-curare would eventually be solved by an examination of the N. O. Menispermaceae and particularly of the genus *Chondodendron.*

The problem of identifying the botanical species employed by the Indians of the Amazon region in the preparation of curare has been admirably clarified by the work of Krukoff and Moldenke.[4] Their definitive study of the American Menispermaceae leaves little to be desired in the way of botanically correlating the various species used as ingredients. They list as important in this respect: *Abuta imene, Chondodendron polyanthum, Ch. limaciifolium, Ch. tomentosum, Ch. ignitanum, Ch. candicans, Telitoxicum minutiflorum, T. peruvianum* and *Abuta rufescens,* all menisperms, and also several *Strychnos* species.



I. *d-Tubocurarine chloride*
$R_1 = CH_3$; $R_2 = H$; of R_3 and R_4, one is H, the other CH_3.
II. *d-Chondocurine dimethochloride*
$R_1 = CH_3$; $R_2 = H$; of R_3 and R_4, one is H, the other CH_3, but in arrangement which is the reverse of that in I.
III. *d-Tubocurarine dimethylether iodide*
$R_1 = R_2 = R_3 = R_4 = CH_3$; anion $I^{=}_2$ instead of $Cl^{=}_2$.

The authenticated plant material collected by Krukoff was investigated chemically and pharmacologically by Folkers.[5] Later, Folkers and Unna[6] reported on the chemical examination of Chazuta curare and its botanical components. As with other species, the crude extract obtained from the dried stem bark of *Chondodendron tomentosum* proved to be highly toxic to frogs and failed to elicit the typical curare response. After separation of the alkaloids into quaternary and non-quaternary fractions, the former caused the characteristic curare symptoms in frogs but was toxic to a cat. The authors, commenting on the close resemblance of the action of this fraction from *Chondodendron tomentosum* to that of native Peruvian curare, cite this fact as supporting evidence for the statement of Krukoff that this plant is the chief ingredient. Folkers was also careful to point out that the Indians extract green fresh bark, whereas the laboratory tests were made with dried bark, and that this may account for the essentially negative results.

On the other hand, the essential ingredients of calabash curare are almost certainly not menispermaceous plants, but members of the *Strychnos* family. This follows from the work of Wieland and collaborators,[7] who isolated from calabash curare several highly active quaternary bases chemically unrelated to the bisbenzylisoquinoline alkaloids, and later demonstrated the presence of some of these compounds in the bark of *Strychnos toxifera.*

We have had the opportunity to examine a sample of curare, prepared by Indians of the upper Amazon, in which only one plant species, namely *Chondodendron tomentosum,* was used. The plant species was identified by a botanist at the time of preparation and authenticated by herbarium specimens. We have been able to isolate from this curare, by procedures which will be described in detail elsewhere, four crystalline tertiary bases and a highly active crystalline quaternary base, which was shown to be identical with the d-tubocurarine of King. In terms of physiological activity the yield of the quaternary alkaloid was 40 per cent.

The tertiary bases, three of which (1, 3 and 4 below) represent isomers of the formula $C_{36}H_{38}O_6N_2$, are:

(1) d-Isochondodendrine, a phenolic alkaloid previously obtained by Scholtz[8] from *pareira brava* and by King[3] from various other *Chondodendron* species.

[2] H. King, *Jour. Chem. Soc.* (London) 1472, 1937.
[3] H. King, *Jour. Chem. Soc.* (London) 737, 1940.
[4] B. A. Krukoff and H. N. Moldenke, *Brittonia,* 3: 1, 1938.
[5] K. Folkers, *Jour. Am. Pharm. Assoc.,* 27: 689, 1938.
[6] K. Folkers and K. Unna, *Arch. Int. Pharmacodyn.,* 61: 370, 1939.
[7] H. Wieland *et al., Ann.* 627: 160, 1937; 536: 68, 1938; 547: 140, 156, 1940.
[8] M. Scholtz, *Arch. Pharm.,* 251: 136, 1913.

(2) d-Isochondodendrine dimethylether, an alkaloid encountered so far only in an asiatic Menisperm, *Cissampelos insularis*.[9] (3) A new alkaloid for which we propose the name *d-chondocurine*. By N-methylation this compound was converted into amorphous quaternary halides (chloride and iodide) which differed chemically and in physiological activity from the corresponding halides of d-tubocurarine. However, on methylation of the phenolic groups in addition to N-methylation it yielded a crystalline dimethylether dimethiodide which was found to be identical with d-tubocurarine dimethylether iodide (III). It must therefore be concluded that d-chondocurine corresponds to d-tubocurarine in regard to the basic ring skeleton and the configuration of the asymmetric centers, but differs from it besides in the valency of the nitrogen atoms, by the arrangement of methylated and free phenolic hydroxyl groups (II). It is noteworthy that the as yet unknown tertiary base, ("d-tubocurine"), corresponding in *all* respects to d-tubocurarine has so far not been encountered by us in *Chondodendron tomentosum* in spite of the relative abundance of the quaternary base in this plant. (4) A new levorotatory alkaloid, differing from l-curine, the tertiary base previously found in curare by Boehm[10] and isolated by King and others from extracts of various *Chondodendron* species. The new alkaloid yielded a crystalline dimethiodide and an amorphous dimethylether dimethiodide. Pending the preparation of larger amounts the question of its chemical relationship to the other alkaloids of this group will have to be left open. There was no evidence for the presence of either l-curine or its enantiomorph, d-bebeerine, in our extract.

In Table 1, the properties of the isolated alkaloids as well as of the quaternary bases and the quaternary dimethylethers prepared from them are recorded. The potency of d-tubocurarine chloride measured by the rabbit head drop method of Holaday,[11] is 6.5 units per mg.[12] The same value is obtained for the (amorphous) iodide of this base after correction for the different atomic weight of the anion. The finding that the quaternary derivative of d-isochondodendrine is practically devoid of lissive action is confirmatory of earlier reports.[13] The more surprising is the fact that the quaternary base derived from d-chondocurine (either in the form of the chloride or the iodide) possesses about three times the lissive potency of d-tubocurarine. Also the crystalline quaternary base corresponding to alkaloid 4 is only slightly less potent than d-tubocurarine. This is, to our knowledge, the first instance where tertiary alkaloids of the bisbenzylisoquinoline type have been shown to yield quaternary bases approximating or exceeding in physiological potency the active constituents of native curare.

TABLE 1

ISOLATED AND DERIVED ALKALOIDS FROM CHONDODENDRON TOMENTOSUM

Tertiary alkaloids	Quaternary alkaloids
d-Isochondodendrine* m.p. 300°; $[\alpha]_D$ + 120° (0.1N HCl)	d-Isochondodendrine dimethiodide m.p. 280°; $[\alpha]_D$ + 87° (water). < 0.4 units per mg.
d-Isochondodendrine dimethylether* m.p. 270°; $[\alpha]_D$ − 15° (chloroform)	d-Isochondodendrine dimethylether dimethiodide m.p. 300°; $[\alpha]_D$ − 7° (ethanol). 1.6 units per mg.
d-Chondocurine* m.p. 234°; $[\alpha]_D$ + 200° (0.1N HCl)	d-Chondocurine dimethiodide ("d-Chondocurarine iodide") amorphous; $[\alpha]_D$ + 178° (methanol). 20 units per mg. dimethylether identical with d-tubocurarine dimethylether iodide.
Alkaloid 4* m.p. 167°; $[\alpha]_D$ − 248° (0.1N HCl)	Dimethiodide of Alkaloid 4 m.p. 250°; $[\alpha]_D$ − 135° (methanol). 5 units per mg. Dimethylether dimethiodide of Alkaloid 4 amorphous 18 units per mg.
Tertiary alkaloid corresponding to d-tubocurarine ("d-tubocurine") unknown.	d-Tubocurarine chloride* m.p. 275°; $[\alpha]_D$ + 225° (water). 6.5 units per mg. d-Tubocurarine dimethylether iodide m.p. 266°; $[\alpha]_D$ + 160° (water). 60 units per mg.

* Alkaloids isolated from *Chondodendron tomentosum;* the other compounds are derivatives prepared in the laboratory. The standard errors for the potency figures given lie within a range of ± 2 to 3 per cent.

The unexpected finding that methylation of the free phenolic hydroxyl groups in all the quaternary bases markedly increased the physiological activity is likewise of interest. In the case of d-tubocurarine, this increase is about nine-fold, and with d-chondocurine dimethiodide, which yields the same dimethylether, three-fold. A similar enhancement of potency (about four-fold) results from the O-methylation of the dimethiodide of alkaloid 4 and, on a considerably lower level of activity, of d-isochondodendrine dimethiodide. O-ethylation of d-tubocurarine has no such marked effect (diethylether iodide, 10 units per mg), while

[9] H. Kondo, M. Tomita and S. Uyeo, *Ber. Dtsch. Chem. Ges.*, 70: 1890, 1937.

[10] R. Boehm, *Abh. Kgl. sächs. Ges. Wiss.*, 22: 203, 1895; 24: 23, 1896; *Arch. Pharm.*, 235: 660, 1897.

[11] H. Holaday, to be published.

[12] The unit referred to its equivalent to 1 mg of an arbitrary curare standard preparation, which was later shown by us to contain likewise d-tubocurarine as the active principle. The potency figures for the other compounds listed in Table 1 were arrived at by comparison with this standard or with crystalline d-tubocurarine chloride. We wish to emphasize that the relative potencies thus determined hold true only when the rabbit is employed as the test animal. When d-tubocurarine dimethylether iodide was compared with the unmethylated base by the same technique in other species (monkey, mouse, dog) the ratios deviated considerably from that obtained in the rabbit. These findings as well as the data incorporated in this paper will be reported in detail by Holaday and associates in a separate communication. We wish to express our sincerest thanks to Mr. H. Holaday of the Biological Laboratories of E. R. Squibb and Sons for placing the bioassay data at our disposal.

[13] M. Scholtz, *Arch. Pharm.*, 252: 513, 1914.

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.