lated by Robison and Morgan[9] from the products of yeast fermentation.

The enzyme has been only partially purified and still contains the enzymes which transform glucose-6-phosphate into glucose-1-phosphate and into fructose-6-phosphate, but the most simple explanation of the chemical changes observed is the equation

UDPG + glucose-6-phosphate ⟶ UDP + trehalose phosphate

(9) R. Robison and W. T. J. Morgan, *Biochem. J.*, **22**, 1277 (1928).

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PATHWAYS OF GLUCOSE CATABOLISM[1]

Sir:

The catabolism of glucose via the Embden–Meyerhof glycolytic pathway would be anticipated to result in the simultaneous contributions to carbon dioxide of carbon atoms 1 and 6 of glucose. By an alternative oxidative pathway via 6-phosphogluconate,[2] known to occur in various biological systems, the appearance of C-1 as carbon dioxide would precede that of C-6.

Glucose-1-C^{14} and glucose-6-C^{14}, the latter kindly supplied by Dr. John C. Sowden, have been compared as precursors of $C^{14}O_2$ when incubated with rat diaphragm sections, kidney slices and liver slices. The experimental conditions were identical with those described.[3] No significant differences in radiochemical yields of $C^{14}O_2$ between the two substrates was noted with diaphragm slices. The ratio

$$\frac{\text{Yield of } C^{14}O_2 \text{ from glucose-6-}C^{14}}{\text{Yield of } C^{14}O_2 \text{ from glucose-1-}C^{14}}$$

is close to unity. With kidney slices, the value of this ratio is approximately 0.9. With liver slices the mean value of this ratio is 0.36.

From studies[3] in which glucose-1-C^{14}, uniformly labeled glucose-C^{14}, lactate-1-C^{14}, lactate-2-C^{14} and lactate-3-C^{14} were compared as precursors of $C^{14}O_2$, no evidence was found supporting the occurrence of a non-glycolytic pathway in rat diaphragm sections. With kidney slices the data suggested the presence of an active non-glycolytic pathway, whereas with liver slices it appeared that the bulk of the carbon dioxide derived from glucose arose by a non-glycolytic route. A quantity, E_{max}, was defined as the maximal contribution of the glycolytic pathway to the over-all conversion of glucose to carbon dioxide. This was calculated to be 0.91, 0.72 and 0.23 for diaphragm, kidney and liver, respectively. These quantities are to be compared with the ratios obtained in the present experiments, and satisfactory agreement is to be noted.

The present experimental approach to the question of the estimation of various pathways of glucose catabolism is simpler than that previously employed and its interpretation requires fewer assumptions.

TABLE I

IN VITRO CONVERSION OF GLUCOSE-C^{14} TO $C^{14}O_2$

Tissues were incubated for 3 hours at 37.8° with 5.5 ml. of bicarbonate buffer containing 50 μM. each of glucose, gluconate, lactate and acetate. The location of the isotope in the labeled glucose is indicated below. Radiochemical yields of $C^{14}O_2$ are calculated per 500 mg. of tissue.

Tissue	Radiochemical yield of CO_2 from glucose, % -1-C^{14}	-6-C^{14}	Ratio $\frac{\text{G-6-}C^{14}}{\text{G-1-}C^{14}}$
Diaphragm	3.76	4.41	1.17
sections	3.79	3.54	0.93
	3.63	3.90	1.07
	3.89	3.56	0.92
Kidney	5.46	5.03	0.92
slices	5.38	5.02	0.93
	5.04	4.38	0.87
Liver	7.64	2.62	0.34
slices	7.19	2.46	0.34
	6.76	2.14	0.32
	10.4	3.76	0.36
	8.49	3.57	0.42

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ALKALOID STUDIES. II.[1] ISOLATION OF RESERPINE AND NARCOTINE FROM *RAUWOLFIA HETEROPHYLLA* ROEM. AND SCHULT.

Sir:

Extracts of the Indian plant *Rauwolfia serpentina* Benth., characterized by an abundance of alkaloids,[2] have been used for some time in India for the treatment of hypertension and other clinical conditions.[3] Acute interest was created by the recent report[4] of the isolation from *R. serpentina* of a crystalline alkaloid, named reserpine, possessing pronounced sedative and hypotensive properties.[5] Several *R. serpentina* extracts of varying degrees of purity are already being employed clinically in this country.

At least one *Rauwolfia species*—*R. heterophylla* Roem. and Schult.—is indigenous to Central and South America and in connection with our present investigations of natural products from Latin American sources it appeared of interest to examine this plant. Such a study seemed especially pertinent because of the report[6] that the Guatemalan *R. heterophylla* ("chalchupa") contains two amorphous alkaloids—chalchupine A and B (m.p. (?) *ca.* 170 and 240°, respectively)—to which were assigned the rather implausible formulas $C_{14}H_{21}N_3O_{12}$ and $C_{15}H_{24}N_6O_{11}$. The presence of the

(1) This work was carried out while Dr. Ben Bloom held a Postdoctoral Fellowship from the Atomic Energy Commission.

(2) B. L. Horecker, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., Vol. I, (1951) p. 117.

(3) B. Bloom, M. R. Stetten and D. Stetten, Jr., *J. Biol. Chem.*, **204**, 681 (1953).

(1) Paper I, C. Djerassi, N. Frick and L. E. Geller, THIS JOURNAL, **75**, 3632 (1953).

(2) *Cf.* A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **36**, 1143 (1953), and references cited therein.

(3) *Inter al.*, M. D. Chakravarti, *Brit. Med. J.*, 1390 (1953).

(4) J. M. Müller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952). No empirical formula for reserpine was established.

(5) H. J. Bein, *ibid.*, **9**, 107 (1953).

(6) E. C. Deger, *Arch. Pharm.*, **275**, 496 (1937).

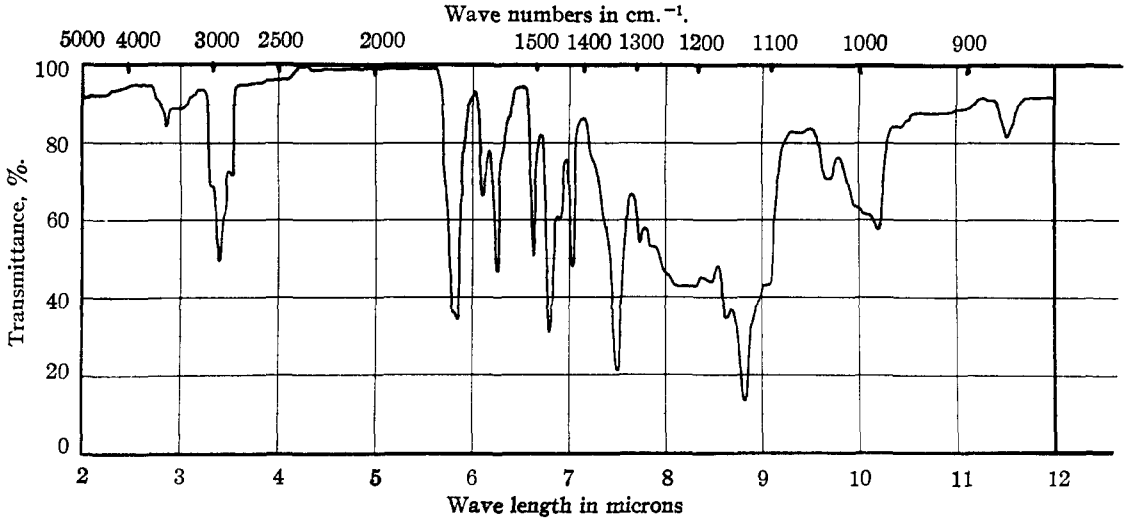

Fig. 1.—Infrared spectrum of reserpine from *Rauwolfia heterophylla* (chloroform solution, 0.1 mm. cell).

"chalchupines" has been corroborated[7] in a study of *R. heterophylla* from Colombia ("piniquepinique") and several pharmacological reports of crude extracts have appeared.[8]

Through the courtesy of Messrs. Mario and Edgar Wunderlich of Guatemala City, we have obtained some authentic *R. heterophylla* from that country while similar material from Mexico was collected by one of us near Oaxaca and identified botanically by Prof. M. Martinez. Chromatography of the benzene-soluble portion of the defatted alcoholic extract of the roots yielded two crystalline alkaloids. The earlier eluted one (m.p. 175–176°, $[\alpha]^{25}D$ −200° ($CHCl_3$), $\lambda_{max.}^{EtOH}$ 292 (3.99), 310 mμ (4.09), $\lambda_{max.}^{CHCl_3}$ 5.62 and 7.93 μ; *Anal.*[9] $C_{19}H_{14}NO_4(OCH_3)_3$ found: C, 63.93; H, 5.66; N, 3.45; methoxyl, 22.12; neut. equiv. ($HClO_4$), 402, Rast mol. wt., 420) was shown to be *l-narcotine* by direct comparison with an authentic specimen of this opium alkaloid kindly supplied by Dr. G. Moersch of Parke, Davis & Company.

The second alkaloid proved to be the widely sought-after *reserpine* (m.p. 262–263°, $[\alpha]^{25}D$ −115° ($CHCl_3$), $\lambda_{max.}^{EtOH}$ 268 mμ (4.15) shoulder at 288–297 mμ (3.95), infrared spectrum in Fig. 1) as demonstrated by direct comparison of the free base and the nitrate with material isolated from the Indian *R. serpentina* and generously furnished by Dr. M. W. Klohs of Riker Laboratories, Inc., and Dr. O. Wintersteiner of the Squibb Institute. We have been able to arrive at a satisfactory empirical formula[4,10] $C_{27}H_{22}N_2O_3(OCH_3)_6$ (Found:[9] C, 65.25; H, 6.42; N, 4.54; methoxyl, 29.83; Rast mol. wt., 619) and if it is assumed that both infrared carbonyl bands at 5.78 and 5.84 μ are due to ester groupings (one of them a methyl ester), then all nine oxygen atoms in reserpine are accounted for. Whether a biogenetic significance can be attributed to the occurrence of both narcotine and reserpine in the same plant must await the structure elucidation of the latter alkaloid. It is noteworthy that the Latin American *R. heterophylla* is the only *Rauwolfia* species other than the Indian *R. serpentina* from which reserpine has so far been isolated.

We are indebted to the Rockefeller Foundation for funds which made possible the plant collections.

JOINT CONTRIBUTION FROM THE
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RECEIVED OCTOBER 14, 1953

(7) R. Paris and R. Mendoza D., *Bull. sci. pharmacol.*, **48**, 146 (1941).

(8) *Cf.* Raymond-Hamet, *Compt. rend.*, **209**, 384 (1939).

(9) Analyses by Mr. J. F. Alicino, Metuchen, N. J.

(10) NOTE ADDED IN PROOF.—Since submission of this paper, three pertinent articles on reserpine have appeared. Our empirical formula is in agreement with that proposed by A. Furlenmeier, *et al.* (*Experientia*, **9**, 331 (1953)) and by N. Neuss, *et al.* (THIS JOURNAL, **75**, 4879 (1953)) but not with that suggested by M. W. Klohs, *et al.* (*ibid.*, **75**, 4867 (1953)). We have confirmed the isolation of trimethylgallic acid from the saponification of reserpine as reported by these three groups.

(11) Pfizer Predoctorate Research Fellow, 1953–1954.

(12) U. S. Public Health Service Predoctorate Research Fellow, 1952–1954.

A REVERSIBLE REACTION OF BOVINE SERUM ALBUMIN

Sir:

We wish to report a reaction of bovine serum albumin (BSA) which has been discovered by a calorimetric procedure.[1] Lowering the *p*H of a BSA solution (ionic strength 0.1 *M*, chloride ion concentration 0.05 *M*) from 4.5 to 3.4 initiates a reaction which absorbs 3,100 cal. per mole of BSA at 25°. The heat absorption follows first order kinetics with high accuracy to more than 90% completion, with a half-time of approximately 2.5 min. The reaction is shown to be completely reversible by the observation that raising the *p*H from 3.4 to 4.5 results in a heat *evolution* of the same magnitude (to within 4%), also following first order kinetics with a half-time of 2.9 min. These heat effects are completely distinguished from the instantaneous heat changes which accompany changes

(1) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **73**, 2454 (1951).