by the unchanged nature of the "pile-up" gradient after withdrawal.

In order to determine the purity of the isolated slow sedimenting fraction, a sample of the removed solution was dialyzed against 0.15 *M* NaCl containing 0.02 *M* sodium phosphate at *p*H 7.40, and then examined in the analytical rotor. The resultant pattern, shown in Fig. 2, displayed the A component and a barely perceptible trace of the faster G component. The former exhibited a corrected sedimentation constant, $s^0_{20} = 4.09\ S$, which agrees with the literature.[6,11]

We have found this technique to be reproducible with respect to the nature of the schlieren patterns of the protein solutions obtained in the preparative tubes, the sampling of the fractions, and the analytical ultracentrifugal purity of the proteins isolated. In addition, such use of the schlieren optical system permits the determination of the location of partly sedimented protein boundaries after preparative angle ultracentrifugation without resort to chemical or biological analysis of isolated fractions. In summary, sampling controlled by observation in the schlieren optical system has a general applicability to colorless as well as colored proteins, and when applied to the slowest sedimenting component of a mixture, is capable of reproducibly yielding isolated proteins of high ultracentrifugal purity by mild physical means.

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INSTITUTE FOR CANCER RESEARCH AND
LANKENAU HOSPITAL RESEARCH INSTITUTE
PHILADELPHIA, PENNA.

(11) See: G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951); G. L. Miller and R. H. Golder, *Arch. Biochem. and Biophys.*, **36**, 249 (1952); J. F. Taylor, *ibid.*, **36**, 357 (1952).

COMMUNICATIONS TO THE EDITOR

THE ENZYMIC SYNTHESIS OF TREHALOSE PHOSPHATE[1]

Sir:

Uridine diphosphate glucose (UDPG)[2] has been found to disappear when incubated with a yeast extract and glucose monophosphate. This disappearance may be measured by estimating UDPG by its coenzymatic activity[3] and also as a decrease in acid-labile glucose. During the reaction UDP is formed and the reducing power of the mixture decreases. As shown in Table I, these changes are equivalent and do not take place when any one of the reactants is added at the end of the incubation period.

TABLE I

ANALYTICAL CHANGES PRODUCED BY THE ENZYME

Incubation of 0.4 μmole of glucose-6-phosphate, 0.6 μmole of UDPG and 0.02 ml. of enzyme in 0.14 *M* tris-(hydroxymethyl)-aminomethane buffer of *p*H 7 during 100 minutes at 37°; total volume, 0.1 ml.; results expressed in μmoles. The enzyme was obtained by disintegrating brewer's yeast cells with sand in a 50 cycles per second oscillator. After centrifuging the supernatant was made 0.5 saturated with ammonium sulfate and the precipitate was dialyzed.

Sample	Substance omitted during incubation[a]	Δ Reducing power[b]	Δ Labile glucose[c]	Δ UDP[d]
1	Glucose-6-phosphate	0	−0.04	+0.02
2	UDPG	0	0	0
3	None	−0.13	−0.14	+0.14

[a] The substance omitted was added at the end of the incubation period. The Δ values represent the difference with sample 2. [b] Calculated as glucose. [c] Hydrolyzed 10 minutes at pH 2 followed by precipitation with zinc sulfate and barium hydroxide. Practically all the glucose liberated under these conditions is that of UDPG. [d] Estimated by a method based on the reaction: phosphopyruvate + UDP → pyruvate + UTP (A. Kornberg, in "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 392). Pyruvate measured colorimetrically.

Samples equal to those shown in Table I were submitted to fractionation of the barium salts. The water-soluble, alcohol-insoluble fractions were used for paper electrophoresis with borate buffer[4] and the phosphate containing compounds were subsequently developed with a molybdate spray reagent.[5] The experiment showed that sample 3, but not samples 1 or 2, contained a phosphate compound which migrated at 60% the rate of glucose-6-phosphate. Dephosphorylation of this compound with kidney phosphatase produced a substance which gave the same R_f value as trehalose when chromatographed on paper.

In other experiments the reaction products were deproteinized by heating, treated with charcoal in order to remove the nucleotides and submitted to the action of phosphatase. When chromatographed on paper a substance migrating like trehalose was found to be present in sample 3 but not in the others. The substance extracted from the paper was hydrolyzed in 1 *N* acid during 3 hours at 100° and compared chromatographically with trehalose treated in the same manner. In both cases a glucose and a trehalose spot were obtained.

The solvent used for paper chromatography was pyridine–ethyl acetate–water[6] with which trehalose, saccharose, maltose and lactose can be separated and the developer was an alkaline silver reagent[7] which reacts slowly with non-reducing disaccharides. Furthermore, reducing from non-reducing sugars can be distinguished because only the latter give color with the aniline-phthalate spray reagent.[8] Thus the ester appears to be a phosphate of trehalose which is presumably identical to that iso-

(1) This investigation was supported in part by a research grant (G-3442) from the National Institutes of Health, U. S. Public Health Service, and by the Rockefeller Foundation.

(2) These abbreviations will be used: UDPG for uridine diphosphate glucose, UDP for uridine diphosphate, and UTP for uridine triphosphate.

(3) R. Caputto, L. F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.*, **184**, 333 (1950).

(4) R. Consden and W. M. Stanier, *Nature*, **169**, 783 (1952).

(5) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(6) M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, **44**, 402 (1949).

(7) W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

(8) S. M. Partridge, *ibid.*, **164**, 443 (1949).

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).

INSTITUTO DE INVESTIGACIONES
BIOQUÍMICAS, FUNDACIÓN CAMPOMAR — L. F. LELOIR
JULIÁN ALVAREZ 1719 — E. CABIB
BUENOS AIRES, ARGENTINA

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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 sections	3.76	4.41	1.17
	3.79	3.54	0.93
	3.63	3.90	1.07
	3.89	3.56	0.92
Kidney slices	5.46	5.03	0.92
	5.38	5.02	0.93
	5.04	4.38	0.87
Liver slices	7.64	2.62	0.34
	7.19	2.46	0.34
	6.76	2.14	0.32
	10.4	3.76	0.36
	8.49	3.57	0.42

DIVISION OF NUTRITION AND PHYSIOLOGY
THE PUBLIC HEALTH RESEARCH INSTITUTE
OF THE CITY OF NEW YORK, INC. — BEN BLOOM
NEW YORK, N. Y. — DEWITT STETTEN, JR.

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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).