unhydrated particles, for which $f/f_0 = 1$, equation (12) is in good agreement with Einstein's theoretical value for these particles[24]

$$[\eta] = 2.5 \qquad (13)$$

Furthermore, equation (12) is supported in the more general case by the data shown in Table II.

TABLE II

FRICTIONAL FACTORS AND INTRINSIC VISCOSITIES OF VARIOUS PROTEINS

Protein	1 f/f^{0a}	2 $[\eta]$ calcd.[b]	3 $[\eta]$ obsd.[a]
Pepsin	1.08	3.2	5.2
Hemoglobin	1.16	4.3	5.3
Egg albumin	1.17	4.5	5.7
Helix pomatia hemocyanin	1.24	5.6	6.4
Serum albumin	1.25	5.8	6.5
Lactoglobulin	1.26	6.0	6.0
Homarus hemocyanin	1.27	6.2	6.4
Amandin	1.28	6.4	7.0
Octopus hemoglobin	1.38	8.7	9.0
Serum globulin	1.41	9.5	9.0
Thyroglobulin	1.43	10.0	9.9
Gliadin	1.60	15.5	14.6
Helix hemocyanin	1.89	30.4	18.0

[a] From a table compiled by Mehl, Oncley and Simha.[15] The values of f/f^0 were calculated from sedimentation and diffusion data by the method of Svedberg and Pedersen.[9] [b] Calculated from values in column 1 by use of equation (12).

Equations (11) and (12) may now be combined to give

$$M^{2/3} = 4.82[\eta]^{1/3} S_0 \eta^0_{20} \pi N (3V_{20}/4\pi N)^{1/3}/(1 - V_{20}\rho^0_{20}) \qquad (14)$$

$[\eta]$ may finally be replaced by its equivalent as given in equation (6) or (8). Molecular weights calculated with either substitution, and also with experimentally determined $[\eta]$, are shown in the last three columns of Table I. The last mentioned method is, of course, the equivalent of Lauffer's method.[20] It is seen that using equation (6) for values of A greater than 8, and equation (8) for values of A under 8, reasonably good approximations of molecular weights are obtained based only on sedimentation rate and partial specific volume data.

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PHILADELPHIA, PENNA.

Utilization of Guanine by *Tetrahymena geleii*[1]

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Tetrahymena geleii has been shown to have an absolute requirement for guanine, although adenine will satisfy a portion of this requirement.[2] It has been assumed, therefore, that all purines of the organism are derived from these exogenous sources. Flavin and Graff showed combined nucleic acid purines to be derived from preformed purines in strain H by administration of labeled guanine[3] and adenine,[4] followed by isolation from the organisms of purines with approximately the same specific activity. Although these workers did not isolate the purines from the acid-soluble nucleotides, the copper precipitate from this fraction was reported to have low activity. This finding suggested the possibility of a synthesis of acid-soluble purines from non-purine precursors, as found by Abrams[5] in the case of a purine-requiring yeast. This was found not to be true for *Tetrahymena* in the studies described below.

Experimental

Guanine-8-C^{14} was prepared by a modification of the Traube[6] method. Three hundred mg. (0.0044 mole) of sodium formate[7] containing 0.6 millicurie of carbon-14 was mixed with 0.94 ml. (0.022 mole) of 90% formic acid. 475 mg. (0.0022 mole) of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride[8] was added, and the mixture refluxed for 24 hours (yields of approximately 75% were obtained after 12 hours). A trap containing sodium hydroxide pellets was placed at the top of the condenser. At the conclusion of the reaction, the excess radioactive formate was recovered by distillation.

The crude guanine was dissolved in N hydrochloric acid, partially decolorized with charcoal, and reprecipitated twice as the free base at *p*H 6, to give 315 mg. (95%) of partially purified product. The guanine was recrystallized twice as the sulfate and twice as the hydrochloride. The product gave a negative phosphotungstic acid test[9] before and after heating in dilute acid, indicating the absence of unreacted triaminohydroxypyrimidine and the intermediate formamidopyrimidine. The white guanine-8-C^{14} hydrochloride (225 mg., 54%, with more recoverable from supernatants) gave the ultraviolet absorption spectrum of pure guanine. Paper strip chromatograms in four solvent systems and ion-exchange chromatography[10] showed only one component, which also contained all the radioactivity.

Culture of Organisms.—*Tetrahymena geleii* W. was grown (4 days, 25°, in the dark) in sterile culture in one liter of medium A[11] modified as follows: Tween 80 (10 mg./ml.) was substituted for Tween 85, 12.4 mg. of guanine-8-C^{14}-HCl (10 μg. guanine/ml.) and 40 μg. uracil/ml. were used in place of the nucleic acid derivatives listed, concentrations of thioctic acid (= protogen) and the other eight vitamins were doubled. The culture was aerated by placing it in a 3.5-gallon Pyrex bottle which was rotated on its side at 10 r.p.m. by two motor-driven rollers. An air inlet and outlet, protected by sterile cotton plugs, were mounted in a swivel joint in the rubber stopper; air was supplied by an aquarium pump. Respiratory carbon dioxide was collected in 2 N sodium hydroxide and precipitated with barium chloride.

Isolations.—The procedures were similar to those previously described,[10] aliquots of various fractions being removed for radioactivity assay. After the medium had been removed from the organisms by settling in the cold, they were washed three times with 1% sucrose in the same manner, and once with water by centrifugation, wet weight *ca.* 10 g. Acid-soluble nucleotides were extracted with three 100-ml. portions of 5% trichloroacetic acid (TCA) in the Waring blendor at 0–5°, and centrifuged cold. This

(1) Supported by a grant from Research Corporation and by Contract No. AT(30-1)-1351 with the U. S. Atomic Energy Commission.

(2) G. W. Kidder and V. C. Dewey, *Proc. Natl. Acad. Sci. U. S.*, **34**, 566 (1948).

(3) M. Flavin and S. Graff, *J. Biol. Chem.*, **191**, 55 (1951).

(4) M. Flavin and S. Graff, *ibid.*, **192**, 485 (1951).

(5) R. Abrams, *Arch. Biochem. Biophys.*, **37**, 270 (1952).

(6) W. Traube, *Ber.*, **33**, 1371 (1900).

(7) Purchased from the U. S. Atomic Energy Commission.

(8) Kindly supplied by Dr. R. B. Angier, Lederle Laboratories, American Cyanamid Co.

(9) H. B. Lewis and B. H. Nicolet, *J. Biol. Chem.*, **16**, 369 (1914).

(10) M. R. Heinrich, V. C. Dewey, R. E. Parks, Jr., and G. W. Kidder, *ibid.*, **197**, 199 (1952).

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fraction was hydrolyzed with sulfuric acid, and copper purines precipitated.[12]

The TCA-extracted tissue was washed twice with cold ethanol, and lipides removed by refluxing three times with 150-ml. portions of 3:1 ethanol–ether, and once with ether; dry weight 385 mg. Combined nucleic acids were obtained by hot sodium chloride extractions, maintaining *p*H 7–8.[13] Nucleates were precipitated with 3 volumes of cold ethanol, and washed with ethanol and ether; weight 39.1 mg.

RNA and DNA were separated by hydrolysis in 2 ml. of *N* sodium hydroxide at room temperature, followed by precipitation of DNA.[14] The DNA precipitate was redissolved in 1 ml. of 0.1 *N* sodium hydroxide, immediately reprecipitated, and washed with TCA. DNA was extracted from the precipitate with hot TCA,[15] the solution made 1 *N* with hydrochloric acid, and hydrolyzed 1 hour at 100°. The solution was evaporated to dryness and a portion used for paper chromatography. The RNA contained in the DNA supernatants (0.1 ml. gave a negative cysteine–sulfuric acid test[16]) was hydrolyzed in *N* hydrochloric acid at 100° for 1 hour, and copper purines precipitated.

Copper purines of the ASN and RNA fractions were separately treated with hydrogen sulfide,[12] and the purine solutions evaporated *in vacuo* several times to remove excess acid. The residues were taken up in 0.5 *N* hydrochloric acid and separated on a column of Dowex-50.[10]

Radioactivity Assays.—All samples were counted with a thin mica window Geiger–Mueller tube. Aliquots of fractions obtained during the isolations were oxidized to carbon dioxide by wet combustion,[17] and barium carbonate plates prepared by filtration on paper. Purine fractions obtained in the ion-exchange separation were often not sufficiently pure for direct combustion and assay. The most satisfactory procedure was preliminary separation of purines on the ion-exchange column, followed by paper chromatography of portions of each purine on 3-cm. strips of Whatman No. 1 paper in Wyatt's[18] isopropyl alcohol–2 *N* hydrochloric acid solvent. Discs 14 mm. in diameter were cut from the purine spots with a sharp cork borer, counted, and eluted with 3 ml. of 0.1 *N* hydrochloric acid. Eluates were read in the Beckman DU spectrophotometer against eluates of discs cut from blank strips run at the same time. The quantity of purine present was calculated from the extinction coefficients of Wyatt,[18] correcting for any non-purine absorption by reading at 300 or 310 mμ.[19]

TABLE I

INCORPORATION OF GUANINE-8-C^{14} IN *T. geleii*

	Specific activity, c.p.m./mg. C[a]	c.p.m./μg. C[b]
Guanine administered	12,700	35.2
Respiratory CO_2, 4 days	0	
Whole cells	15	
Medium	1	
Acid-soluble compounds[c]	18	
Lipide-free tissue	186	
Combined nucleic acids	1000	
Nucleic acid-free tissue	2	
ASN guanine		33.8
ASN adenine		30.8
ASN hypoxanthine[d]		27.2
RNA guanine		31.2
RNA adenine		31.3
DNA guanine		31.2
DNA adenine		29.8

[a] Counts/min./mg. carbon, counted as barium carbonate, corrected to infinite thickness. [b] Counts/min./μg. carbon, counted on filter paper discs; this procedure results in higher specific activities than those obtained with barium carbonate plates of the same area. [c] Cold TCA extract after removal of TCA by ether extraction. [d] Arising principally by enzymatic deamination of adenine during isolation.

Results

The data of Table I show that guanine-8-C^{14} was utilized to the same extent for all the purines of the various fractions, within the accuracy of the method. It is evident that *T. geleii* W. utilizes exogenous purine exclusively, and does not synthesize purine from smaller precursors; such synthesis would dilute the activity of the guanine administered. As further evidence for lack of synthesis, no activity was found in ASN purines, total nucleic acids or on paper strips of hydrolyzed RNA and DNA after giving 30 mg. of sodium formate-C^{14} (31,000 counts/min./mg. carbon). Low activities were found in respiratory carbon dioxide during the five-day growth period.

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The Syntheses of 5- and 6-Chloroacetovanillone[1]

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During a study of the oxidation of chlorite lignin with nitrobenzene and alkali, 6-chlorovanillin and 6-chlorovanillic acid were isolated.[3] In anticipation of the isolation of chloro analogs of other products of the nitrobenzene oxidation of lignin, the synthesis of 5- and 6-chloroacetovanillone were undertaken.

Attempted condensation of a methyl Grignard reagent with 6-chlorovanillonitrile acetate was unsuccessful, as were the many attempted oxidations of 1-(6-chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol, a compound prepared by the condensation of 6-chlorovanillin with methylmagnesium iodide. Recourse was then had to the diazomethane method for the synthesis of acetophenones from benzaldehydes.

Reaction of 6-chlorovanillin acetate with diazomethane yielded as the main product the β-hydroxyketone formed from two moles of the aldehyde with one mole of diazomethane, namely, 1,3-bis-(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone (I). Deacetylation under mild alkaline conditions caused dehydration and yielded 6,6′-dichloro-4,4′-dihydroxy-3,3′-dimethoxychalcone (II). The β-hydroxyketone (I) was dehydrated to the diacetate of the chalcone (II) by means of acetic anhydride in pyridine. Complete hydrolysis of I yielded 6-chlorovanillin and the desired 6-chloroacetovanillone. 5-Chloroacetovanillone was prepared in similar fashion by hy-

(1) A portion of a thesis submitted to Lawrence College in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy. This work was carried out under the direction of I. A. Pearl.
(2) Kimberly–Clark Corp., Neenah, Wisconsin.
(3) J. E. Jayne, Dissertation, The Institute of Paper Chemistry, 1953.