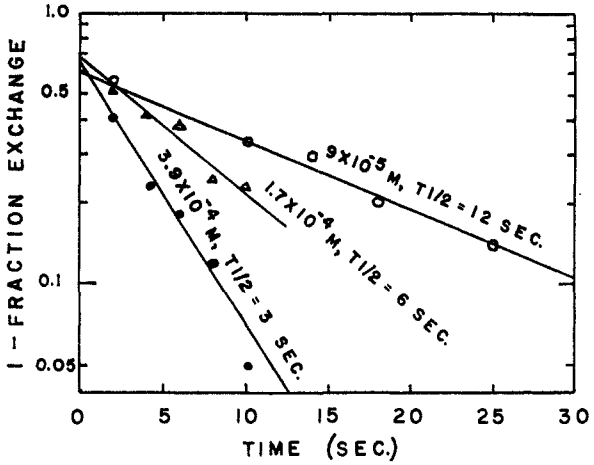

Fig. 1. Exchange curves 1°, 0.15*f* NaOH: concentrations are total manganese concentrations; $(C_6H_5)_3$ Br extraction separation used in the run at the intermediate concentration; $(C_6H_5)ReO_4$ coprecipitation separation used in the other two runs

tration is consistent with a second-order rate law with a constant of 650 M^{-1} sec.$^{-1}$.

In 2 *f* NaOH the rate of exchange is approximately twice that in 0.15 *f* NaOH, and the zero-time exchange is also greater. These observations are consistent with the nearly complete exchange in 15 seconds observed by Bonner and Potratz[4] for similar experimental conditions.

The fact that the rate of the exchange is small compared to collision frequencies shows that in aqueous solution the probability of electron transfer from $MnO_4^=$ to MnO_4^- is small, even though for these symmetrical reactants the Franck–Condon type restrictions are minimal.[5]

We hope to improve our technique sufficiently to make a detailed kinetic study of this exchange reaction.

(5) W. F. Libby, *J. Chem. Phys.*, **56**, 863 (1952).

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RECEIVED SEPTEMBER 14, 1953

REFRACTIVE INCREMENT OF THYMUS NUCLEIC ACID

Sir:

Recently we have had occasion to question the numerical value (0.160 ml.-g.$^{-1}$) of the refractive increment, d*n*/d*c*, of thymus nucleic acid (DNA), as published by Tennent and Vilbrandt in THIS JOURNAL[1] and cited by Smith and Sheffer,[2] Katz,[3] and Doty and Bunce.[4] We have measured the d*n*/d*c* of two samples of DNA prepared in our laboratory from fresh thymus tissue. Sample No. 1 was prepared by the method of Mirsky and Pollister[5]; the nucleoprotein of sample No. 2 was prepared in the same way as sample No. 1, but was deproteinized using the dodecyl sulfate denaturation described by Marko and Butler.[6] The samples were dissolved in the desired salt solution, stirred for at least 24 hours, and then dialyzed for several days against the salt solution, the dialysate being replaced occasionally.

The difference Δn in index of refraction between the DNA solution and the last dialysate was in each case measured at 20°, using 4358 Å. light, by means of a differential refractometer. This instrument was somewhat similar to that described by Brice and Halwer[7] and was calibrated using data given by Stamm.[8] Using this calibration, a value of 0.178 ml.-g.$^{-1}$ was obtained for d*n*/d*c* of tobacco mosaic virus.

The concentration of each DNA sample was determined from its optical density at 2600Å. (measured by dilution into 0.1 *M* acetate buffer, *p*H 4.3), the ratio of density to phosphorus content for the sample and the per cent. phosphorus in DNA. For sample 1, the ratio of optical density (in 0.1 *M* acetate buffer, *p*H 4.3) in a 1-cm. cell to phosphorus was 6620 per mole of phosphorus/liter, and 6640 for sample 2. From data given by Sinsheimer and Koerner[9] on the nucleotide composition of DNA, it was computed that 9.35% by weight of the sodium salt of DNA is phosphorus.

Results are listed in the table:

DNA prepn.	Solvent	Δn	Concn. in g./ml.	$\Delta n/c$, ml.-g.$^{-1}$
1	0.1 *M* sodium acetate + 0.2 *M* sodium chloride, *p*H 5.7	0.701×10^{-4}	0.347×10^{-3}	0.202
	0.05 *M* sodium chloride	0.638×10^{-4}	0.323×10^{-3}	0.198
	0.001 *M* sodium chloride	0.599×10^{-4}	0.298×10^{-3}	0.201
2	0.1 *M* sodium acetate + 0.2 *M* sodium chloride, *p*H 5.7	1.63×10^{-4}	0.821×10^{-3}	0.199
	0.05 *M* sodium chloride	5.30×10^{-4}	2.62×10^{-3}	0.202
	0.001 *M* sodium chloride	1.68×10^{-4}	0.827×10^{-3}	0.204
			Av.	0.201

The combined effect on the observed Δn of Donnan equilibrium and of the binding of NaCl by the DNA was computed from data given by Shack, Jenkins and Thompsett[10] for the case where the solvent was 0.05 *M* NaCl. It was found that because of these two effects, the observed Δn of 5.30×10^{-4} may be too large by 1.9%. In making this calculation, it was necessary to assume that the polarizability of NaCl bound to the DNA is the same as that in a free solution of NaCl. For the other solvents the effect of salt binding and Donnan equilibrium cannot be calculated from the work of Shack, *et al.*, since they did not go to as low a NaCl concentration as 0.001 *M*, nor did they investigate the acetate buffered NaCl solution.

Since d*n*/d*c* enters to the second power in light-

(1) H. G. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 424 (1943).
(2) D. B. Smith and H. Sheffer, *Can. J. Research*, **B28**, 96 (1950).
(3) S. Katz, THIS JOURNAL, **74**, 2238 (1952).
(4) P. Doty and B. H. Bunce, *ibid.*, **74**, 5029 (1952).
(5) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 117 (1946).
(6) A. M. Marko and G. C. Butler, *J. Biol. Chem.*, **190**, 165 (1951).
(7) B. A. Brice and M. Halwer, *J. Opt. Soc. Am.*, **41**, 1033 (1951).
(8) R. F. Stamm, *ibid.*, **40**, 788 (1950).
(9) R. L. Sinsheimer and J. F. Koerner, *J. Biol. Chem.*, **198**, 293 (1952).
(10) J. Shack, R. J. Jenkins and J. M. Thompsett, *J. Biol. Chem.*, **198**, 85 (1952).

scattering determinations of molecular weights, the molecular weights of DNA as determined by Smith and Sheffer, Katz, and Doty and Bunce would seem to be too high by 37%. It should be pointed out that Tennent and Vilbrandt did not specify the wave length light used in their refractive measurements; hence direct comparison of their value of 0.160 with the above value of 0.201 should not be made.

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RECEIVED JULY 24, 1953

VALINE BIOSYNTHESIS IN *TORULOPSIS UTILIS*[1]

Sir:

Results are reported herein which indicate that the carbon chain of lactic acid is a direct precursor of the valine carbon skeleton. The materials for this investigation were specimens of labeled valine isolated, by slight modifications of the method of Moore and Stein,[2] from hydrolysates of yeast grown in the presence of C^{14}-labeled tracer substances. Growth of the cells and other experimental details have been described previously.[3] Submission of the valines to a degradation procedure for radioactivity assay of each of the four different valine carbons gave the results shown in the table. Glycine, acetate, and lactate carboxyl carbons appeared only in the valine carboxyl; glycine and acetate α-carbons appeared approximately equally in all of the valine non-carboxyl carbons; and the lactate α-carbon appeared equally and nearly exclusively in carbons 2 and 3 of valine. The relatively low incorporation of acetate and glycine carbons precluded these substances, as well as citric acid cycle components, as direct precursors of valine. However, the relatively high incorporation of lactate carbons suggested that lactate or pyruvate may be the direct source of carbons for valine biosynthesis, and that acetate and glycine carbons were incorporated in valine *via* their prior conversion to pyruvate. The observed distribution of activity is in accord with the conversion of glycine to pyruvate *via* serine, and of acetate to pyruvate *via* the citric acid cycle and oxalacetate. If this postulation is correct, it follows that the methyl carbon of pyruvate should be the precursor of the valine methyl carbons. Indirect proof that carbon 3 of pyruvate can provide the carbon for the valine methyl carbons was obtained in the last experiment in the table in which it was found that carbon 1 of glucose, presumably *via* 3-labeled pyruvate, appeared preponderantly in the methyl carbons of valine.

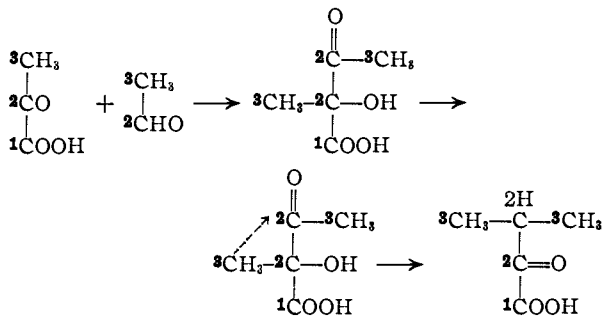
DISTRIBUTION OF LABELED CARBONS IN VALINE CARBON

Values are specific activities in cpm of $BaCO_3$, corrected for equal initial activities of substrates.

Valine carbon number[a]	Precursors: Acetate CH_3–	Acetate –COOH	Glycine $-CH_2-$	Glycine –COOH	Lactate –CHOH–	Lactate –COOH	Glucose-1-C^{14} –CHO
1	105	170	30	575	45	905	25
2	155	−3	365	−4	750	−5	45
3	150	0	310	0	700	} 5[b]	50
4,4'	155	0	335	0	20	}	358

[a] Numbering begins with valine carboxyl carbon. [b] Acetone not further degraded.

In speculating on the mechanism of this conversion, the equal incorporation of lactate carbon 2 into valine carbons 2 and 3 suggests a direct coupling of 2 lactate α-carbons. The only conceivable biological reaction of similar type is the condensation of pyruvate and acetaldehyde to yield acetolactic acid.[4] From the structure of this substance it is not unreasonable to assume that migration of a methyl group might occur, as in the pinacol or related rearrangements, to yield β,β'-dimethylpyruvic acid, a logical precursor of valine. Some precedent for the biological occurrence of methyl group migration has recently been provided by Woodward and Bloch.[5] This pathway is under further investigation.



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RECEIVED AUGUST 14, 1953

(1) Aided by grants from the Atomic Energy Commission, contract No. AT(30-1)777; the American Cancer Society; and the National Cancer Institute of the Department of Health, Education and Welfare.

(2) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

(3) M. Strassman and S. Weinhouse, THIS JOURNAL, **74**, 1726 (1952).

(4) T. P. Singer and J. Pensky, *Biochim. et Biophys. Acta*, **9**, 316 (1952).

(5) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953).

(6) Postdoctoral Fellow of the National Institutes of Health, Department of Health, Education and Welfare.

REARRANGEMENT OF THE STEROID C/D RINGS. SYNTHESIS OF AN 11-KETO-$\Delta^{13(17a)}$-C-*NOR*/D-*HOMO*-STEROID

Sir:

Hecogenin (I) in the form of its toluene *p*-sulfonylhydrazone derivative (Ia), m.p. 259–60° (dec.); found: S, 5.39; $\lambda_{max.}^{CH_3OH}$ 226 mμ (4.1), was submitted to a Bamford–Stevens rearrangement[1] with sodium in ethylene glycol to yield the C-*nor*/D-*homo*-sapogenin (II) m.p. *ca.* 110°; found: C, 77.95; H, 10.00. *Acetate* (IIa) m.p. 142–144°; $[\alpha]^{23}D$ −52.6 ($CHCl_3$). Found: C, 76.03; H, 9.72. II was found to be identical with a companion olefin isolated together with III from the solvolytic rearrangement of the rockogenin derivative (IV)[2]; II was also formed in good yield from III on treatment of the latter with formic acid at room temperature. The endocyclic olefin (II)

(1) W. R. Bamford and T. S. Stevens, *J. Chem. Soc.*, 4735 (1952).

(2) R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, THIS JOURNAL, **74**, 2693 (1952).