high temperature is reached. Using specially treated 2-liter Pyrex or Vycor flasks we succeeded in reducing the extent of surface decomposition enough to follow the over-all reaction up to 600° at low pressures.[1] The present results are too scanty for an adequate discussion of possible mechanisms although certain chain-breaking processes immediately come to mind.

We have also confirmed the observation of McLane[2] regarding the catalytic action of hydrogen peroxide on the slow hydrogen-oxygen reaction above the second limit; under certain conditions there were indications of rapid self-heating of the mixture prior to explosion and, in other cases, of slow pressure decrease afterward, as in "afterburning." All these effects are being investigated systematically and the results will be published elsewhere.

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DEOXYCYTIDINE DIPHOSPHATE CHOLINE, A NEW DEOXYRIBOSIDIC COMPOUND

Sir:

Our previous report[1] described the presence of "masked" deoxyribosidic compounds in the acid soluble extracts of sea urchin eggs and several other tissues including microörganisms and mammalian viscera. The present communication deals with the further characterization of one of the "masked" deoxyribosidic compounds of sea urchin eggs, which has now been identified as deoxycytidine diphosphate choline, a new deoxynucleotide derivative.

The acid soluble deoxyribosidic compounds were chromatographed on a column of Dowex-1 (X-2, formate) as previously described.[1] The fraction under consideration eluted by 0.1*M* AM-F[2] (*p*H 4.5) (Fraction No. 5 of the previous report) was rechromatographed on the same resin, employing formic acid as an eluent, and the fractions having a CMP-like spectrum were pooled, neutralized, and again applied to a column of Dowex-1 (X-8, formate). The column was then eluted by gradient elution with AM-F (*p*H 7.8) containing sodium tetraborate to yield two separate fractions, designated F_1 and F_2, both having a spectrum similar to CMP. In this system, F_1 was eluted slightly faster than F_2 and the both emerged from the column much more rapidly than authentic deoxy-CMP and CMP. They were further purified by paper chromatography (solvent system 0.02 *N* acetic acid in 60% ethanol[3]), and analyzed for their base, deoxyriboside, phosphorus and choline contents (Table I).

Only after the venom digestion[1] does F_1 become active toward *Lactobacillus acidophilus* R-26, the deoxyriboside requiring organism.[5] Hydrolysis of F_1 and F_2 with 1 *N* HCl for 15 minutes at 100°, followed by paper chromatography yielded deoxy-CMP and CMP, respectively. After the venom digestion, F_1 gave deoxycytidine and choline, F_2 cytidine and choline, identified by paper chromatography. The presence of choline residues in F_1 and F_2 is in harmony with the fact that, in anion exchange chromatography at *p*H 7.8, F_1 and F_2 behaved less anionic than deoxy-CMP and CMP in spite of their high phosphorus contents (Table I). In the microbiological determination of choline, it was noticed that F_1 as well as F_2 did not support the growth of a choline-less mutant of *Neurospora* until after digestion with snake venom, the situation being very similar to that seen in the microbiological determination of deoxyriboside of F_1.

TABLE I

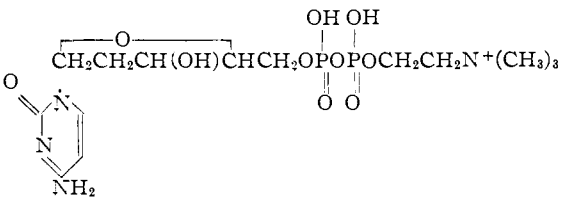
ANALYTICAL DATA OF F_1 AND F_2

	μmole/μmole deoxycytidine or cytidine[a]				
	Deoxyriboside[b] Venom treatment		Phosphorus[c]	Choline[d] Venom treatment	
	Before	After		Before	After
F_1	0.15	0.93	1.93	0	0.83
F_2	0	0	2.03	0	0.87

[a] Deoxycytidine or cytidine moiety identified by characteristic ultraviolet spectrum in acid and alkali. Estimated from 260 mμ absorption at *p*H 2 using $\epsilon = 6200$.[4] [b] Determined microbiologically.[5] Venom treatment was the same as that previously described.[1] [c] Determined by the method of Fiske–SubbaRow.[6] [d] Determined microbiologically.[7] Venom treatment was the same as that used for deoxyriboside determination.[1]

The action of snake venom, which results in the liberation of deoxycytidine and choline, can be attributed to its nucleotide pyrophosphatase and 5′-nucleotidase activity. This view coincides well with the recent report of Schneider and Potter,[8] who demonstrated that di- or triphosphates of pyrimidine deoxyribosides do not support growth of *L. acidophilus* R-26 unless they are dephosphorylated to monophosphates by acid hydrolysis.

From the analytical data shown in Table I and several other lines of evidence mentioned above, it is concluded that F_1 is deoxycytidine diphosphate choline



and F_2 cytidine diphosphate choline, which was discovered previously by Kennedy and Weiss.[3,9]

Recently, Potter[10] reported the occurrence of similar compounds in calf thymus and described

(1) Y. Sugino, N. Sugino, R. Okazaki and T. Okazaki, *Biochim. Biophys. Acta*, in press.

(2) The following abbreviations are used: AM-F = ammonium formate, CMP = cytidylic acid, CDP = cytidine diphosphate.

(3) E. P. Kennedy, *J. Biol. Chem.*, **222**, 185 (1956).

(4) G. H. Beaven, E. R. Holiday and E. A. Johnson, "The Nucleic Acids" (edited by E. Chargaff and J. N. Davidson), Academic Press Inc., Publ., New York, N. Y., 1955, Vol. I, pp. 493–553.

(5) E. Hoff-Jørgensen, *Biochem. J.*, **50**, 400 (1952); "Recent Developments in Cell Physiology" (edited by J. A. Kitching), Butterworth Scient. Publ., London, 1954, pp. 79–90.

(6) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(7) N. H. Horowitz and G. W. Beadle, *ibid.*, 325 (1943).

(8) W. C. Schneider and R. L. Potter, *Proc. Soc. Expt. Biol. Med.*, **94**, 798 (1957).

(9) E. P. Kennedy and S. M. Weiss, THIS JOURNAL, **77**, 250 (1955); *Federation Proc.*, **14**, 234 (1955).

(10) R. L. Potter and V. Buettner-Janusch, *ibid.*, **16**, 234 (1957).

them as deoxy-CDP-X and CDP-X, but the nature of X remained uncertain although it was noted that it was basic.

I am indebted to Dr. Reiji Okazaki of Nagoya University for preparing lyophilized samples of unfertilized sea urchin eggs.

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CORRELATION OF CARBANION REACTIVITIES BY σ_R^- PARAMETERS[1]

Sir:

The definition of nucleophilic resonance parameters, σ_R^-, has been made recently according to the equation[2]

$$\sigma_R^- = \sigma^- - \sigma_I \qquad (1)$$

where σ^- is the dual Hammett sigma value which pertains to the reactions of *p*-substituted derivatives of aniline and phenol,[3] and σ_I is the inductive substituent constant.[2] The σ_R^- values provide a scale of the powers of substituent groups to delocalize negative charge by conjugation.

Useful applicability of the σ_R^- scale to certain nucleophilic reactivities is suggested by the correlation of the rates of carbanion formation of substituted methanes in water at 25° by the equation

$$\log k_1 = (26.0)\sigma_R^- + (4.0)(\Sigma\sigma_I) - 24.8 + \log n_H \qquad (2)$$

The term $\log n_H$ is a statistical correction term, n_H being the number of ionizable H atoms. The rate constants, k_1, are those tabulated by Pearson and Dillon.[4] The correlation, shown in Fig. 1, holds over a spread of rates of eight powers of ten with an average deviation of 0.25 log unit. This is quite satisfactory since the precision of σ_R^- values is no better than ±0.03. Figure 1 shows a plot of $\log (k_1/n_H)$ *vs.* the quantity $(26.0)\sigma_R^- + (4.0)\Sigma\sigma_I$. The full line is one of unit slope.

The term $(4.0)\Sigma\sigma_I$ presumes to measure the inductive contribution, and the term $(26.0)\sigma_R^-$, the resonance contribution to the logarithm of the ionization rate. The enormous (and therefore rather crude) constant for susceptibility to resonance interaction, 26.0 (compared to 4.0 for susceptibility to inductive interaction), indicates that a much greater stabilization by resonance than inductive interaction results from the substitution of conjugated groups directly at the carbanion carbon compared to the corresponding interactions of these groups acting through the ring system of *p*-substituted benzene derivatives.

Equation (2) is unique in that resonance parameters determined from reactivities in the aromatic series are used to correlate the effects of corresponding substituents in the aliphatic series. This usage completes a cycle wherein inductive effects from the aliphatic series (σ_I parameters) have been used to evaluate from aromatic series reactivities (equation (1)), the resonance parameter, σ_R^-. This parameter is now applied to the correlation of resonance effects in the aliphatic series.

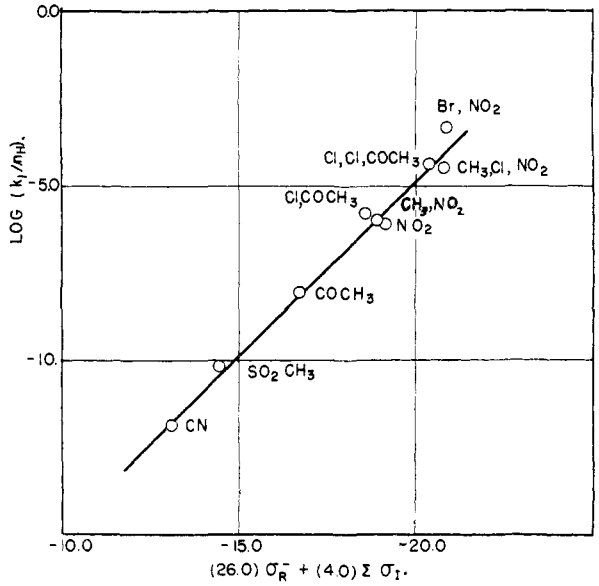

Fig. 1.—Correlation by equation (2) of rates of carbanion formation: $X_1X_2X_3CH + H_2O \longrightarrow [X_1X_2X_3C]^- + H_3O^+$, where X_1 is as indicated and $X_2 = X_3 = H$ unless otherwise indicated. A line of unit slope is shown.

It has been assumed in using equation (2) that σ_R^- values for the substituents Br, Cl, and CH_3 (in the presence of a single conjugating group such as NO_2, CH_3CO, CH_3SO_2, or CN) are zero, and that the former substituents contribute only to the $\Sigma\sigma_I$ term. If equation (2) is applied to polysubstitution of conjugating substituents (using $\Sigma\sigma_R^-$) substantial deviations are obtained (Table I lists some typical deviations).

TABLE I

DEVIATIONS FROM EQUATION (2) OF RATES OF IONIZATION OF POLYSUBSTITUTED METHANES WITH MORE THAN ONE CONJUGATING GROUP

Substituted methane	$\log(k/n_H)$ exptl.	$\log(k/n_H)$ calcd. eqn. (2)	Deviation log units
$CNCH_2CN$	−0.3	+ 1.2	+ 1.5
$CH_3COCH_2COCH_3$	−0.3	+ 8.6	+ 8.9
$CH_3COCH(Br)COCH_3$	−0.3	+10.4	+10.7
$CH_3COCH(CH_3)COCH_3$	−2.8	+ 8.4	+11.2
$CH_3COCH_2NO_2$	0.0	+11.0	+11.0
$NO_2CH_2NO_2$	+1.4	+13.5	+12.1

It is apparent from Table I that steric inhibition of resonance (and possibly other steric effects) of the second by the first conjugated substituent (and by bulky substituents, *e.g.*, Br, CH_3) contributes appreciably to the failure of equation (2). For example, the rate of ionization of dinitromethane is twelve powers of ten slower than predicted by equation (2). On the other hand, the

(1) This work was supported in part by the Office of Naval Research Project NRO55-328. Reproduction in whole or in part is permitted for any purpose of the United States Government.

(2) R. W. Taft, Jr., THIS JOURNAL, **79**, 1045 (1957); *cf.* also M. S. Newman, "Steric Effects in Organic Chemistry," John Wiley and Sons, Inc., N. Y., 1956, p. 578, 594, and J. D. Roberts and W. T. Moreland, Jr., THIS JOURNAL, **75**, 216 (1953).

(3) *Cf.* (a) reference (2); (b) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 184; (c) H. H. Jaffé, *Chem. Revs.*, **53**, 191 (1953).

(4) R. G. Pearson and R. L. Dillon, THIS JOURNAL, **75**, 2439 (1953). These authors have noted qualitatively the correlation given by equation (2) *cf.* footnote (35).