e.g., it gives no pyrazole derivative with hydrazine and no color with ferric chloride,[1b] and (2) the diol IV produced by the action of lithium aluminum hydride on tetrahydroalantolactone affords a dibenzoate, but that obtained from desoxytetrahydrosantonin gives only a monobenzoate.[1d]

These facts led us to question the position of the lactone ring in the formula (I) suggested by Ruzicka, *et al.*, for alantolactone.[4,5] The following experiments have enabled us to conclude that alantolactone should be represented by II and not I.

The keto ester V,[1b,6] b.p. 134–135° (0.05 mm.), $[\alpha]^{25}D$ −26° (c = 5, $CHCl_3$), was treated with methylmagnesium iodide to give 7-methyltetrahydroalantolactone (VI), in 44% yield, m.p. 99–100°, $[\alpha]^{25}D$ −0.8° (c = 5, $CHCl_3$), Calcd. for $C_{16}H_{26}O_2$: C, 76.75; H, 10.47. Found: C, 76.72; H, 10.54, together with VII, in 6% yield, m.p. 199–200°, $[\alpha]^{25}D$ + 7.0° (c = 2.9, $CHCl_3$), Calcd. for $C_{18}H_{34}O_2$: C, 76.54; H, 12.13. Found: C, 76.16; H, 11.83. The structure of VI is based on analysis, infrared spectrum (ν_{max} 1762 cm.$^{-1}$, γ-lactone), and the facts that it is converted to an acid by methanolic alkali and recovered unchanged in acidic medium, and that reduction with lithium aluminum hydride yielded a diol (VIII), m.p. 157–158°; calcd. for $C_{16}H_{30}O_2$: C, 75.53; H, 11.89. Found: C, 75.20; H, 11.84.

Dehydrogenation of VI with selenium gave a hydrocarbon which showed characteristic absorption for substituted naphthalenes in the ultraviolet region. Its picrate and styphnate melt at 102–103° and 149°, respectively. The infrared absorption spectrum of this hydrocarbon resembles closely that of 2,3,5-trimethylnaphthalene,[7] so we have synthesized 2,5-dimethyl-3-ethylnaphthalene to compare with the above hydrocarbon.

o-Tolylacetyl chloride was condensed with ethylzinc iodide to give *o*-methylbenzyl ethyl ketone, b.p. 121° (10 mm.) (semicarbazone, m.p. 173–174°), which was converted, by condensation with ethyl α-bromopropionate, followed by dehydration and hydrogenation, into ethyl γ-*o*-tolyl-α-methyl-β-ethyl butyrate, b.p. 160–161° (12 mm.); calcd. for $C_{16}H_{24}O_2$: C, 77.37; H, 9.74. Found: C, 77.49; H, 9.43. Ring closure of the ester with sulfuric acid gave 2,5-dimethyl-3-ethyl-1,2,3,4-tetral-1-one, b.p. 169–170° (10 mm.) (2,4-dinitrophenylhydrazone, m.p. 205–206°; calcd. for $C_{20}H_{22}O_4N_4$: C, 62.81; H, 5.80; N, 14.65. Found: C, 63.15; H, 5.70; N, 14.66). The dimethylethyltetralone was reduced with lithium aluminum hydride to give 2,5-dimethyl-3-ethyl-1,2,3,4-tetral-1-ol, m.p. 101–102°; calcd. for $C_{14}H_{20}O$: C, 82.30; H, 9.87. Found: C, 82.40; H, 9.90, which was dehydrogenated with selenium to 2,5-dimethyl-3-ethylnaphthalene, b.p. 120–121° (1.5 mm.); calcd. for $C_{14}H_{16}$: C, 91.25; H, 8.75. Found: C, 91.28; H, 8.54. Picrate, m.p. 102–103°; calcd for $C_{20}H_{19}O_7N_3$: C, 58.11; H, 4.36; N, 10.17. Found: C, 58.19; H, 4.55; N, 10.18. Styphnate, m.p. 149°; Calcd. for $C_{20}H_{19}O_8N_3$: C, 55.94; H, 4.46; N, 9.79. Found: C, 55.71; H, 4.42; N, 9.81.

(5) L. Ruzicka and P. Pieth, *Helv. Chim. Acta.*, **14**, 1090 (1931).
(6) T. Ukita and S. Nakazawa, *Pharm. Bull.*, **2**, 299 (1954).
(7) W. L. Mosby, THIS JOURNAL, **74**, 2564 (1952).

The synthetic hydrocarbon was identical with that obtained from III as shown by infrared absorption spectrum and by mixed melting points of their picrates and styphnates.

Alantolactone, isoalantolactone, and dihydroisoalantolactone which are isolated from the roots of *Inula helenium* are catalytically hydrogenated to give the same tetrahydroalantolactone,[4,5] and thus the last two lactones must also have oxygen at C_7.

INSTITUTE OF APPLIED MICROBIOLOGY
UNIVERSITY OF TOKYO — KYOSUKE TSUDA
HONGO, TOKYO, JAPAN
TAKAMINE RESEARCH LABORATORY
SANKYO CO. LTD. — KATSUMI TANABE
SHINAGAWA, TOKYO, JAPAN — ISSEI IWAI
PHARMACEUTICAL INSTITUTE
UNIVERSITY OF KYUSHU — KAZUHISA FUNAKOSHI

RECEIVED DECEMBER 28, 1956

OXIDATION STATE OF RESPIRATORY CARRIERS AND THE MECHANISM OF OXIDATIVE PHOSPHORYLATION

Sir:

To account for coupling of phosphorylation to the electron transport chain in mitochondria, it has been proposed that certain oxido-reduction steps lead to the formation of a "high-energy" linkage between a respiratory carrier and either phosphate or some other compound with which phosphate may exchange.[1,2,3,4,5] Such a "high-energy" complex is postulated as the ultimate donor of phosphate to ADP.[6] Thermodynamic considerations alone do not suffice to determine whether such a "high-energy" complex involves the oxidized or the reduced state of the carrier.[5] In this communication evidence is presented that the primary "high-energy" linkage generated during electron transport involves or is dependent upon the oxidized state of the respiratory carriers.

The experiments were carried out with a complex of enzymes which catalyzes electron transport from β-hydroxybutyrate to oxygen and the coupled phosphorylations.[7] Such preparations also catalyze exchange of labeled inorganic phosphate with the terminal phosphate of ATP in the absence of *net* electron transport, a reaction which is a reflection of the activity of the coupling mechanisms.[3,8] Under certain conditions the oxidation–reduction state of the carriers determines the rate of the ATP-P_i^{32} exchange. The exchange is maximal when the carriers are in the oxidized state and is greatly inhibited in the reduced state. Data in Table I show that when the enzyme complex is amply supplied with oxygen, in the absence of added oxidizable substrate, the incorporation of P_i^{32} into ATP is maximal; DNP[6] completely inhibits the reaction. Insignificant *net* electron flux oc-

(1) E. C. Slater, *Nature*, **172**, 975 (1953).
(2) A. L. Lehninger, *Harvey Lectures*, **49**, 176 (1953–1954).
(3) C. Cooper and A. L. Lehninger, *J. Biol. Chem.*, in press (February, 1957).
(4) P. D. Boyer, A. B. Falcone and W. H. Harrison, *Nature*, **174**, 401 (1954).
(5) B. Chance and G. R. Williams, *Adv. in Enzymology*, **XVII**, 65 (1956).
(6) Abbreviations: ATP and ADP, adenosine tri- and diphosphate; P_i, inorganic orthophosphate; DNP, 2,4-dinitrophenol.
(7) C. Cooper and A. L. Lehninger, *J. Biol. Chem.*, **219**, 489 (1956).
(8) C. Cooper and A. L. Lehninger, *ibid.*, in press (February, 1957).

curred since replacement of ATP with ADP yielded negligible uptake of P^{32}. On the other hand, when the enzyme preparation was incubated under strictly anaerobic conditions with β-hydroxybutyrate, the exchange was greatly inhibited. Difference spectra of the respiratory carriers[7] revealed that under the anaerobic conditions described the respiratory carriers were essentially completely reduced. Maintenance of the reduced state by addition of cyanide to inhibit cytochrome oxidase similarly led to almost complete inhibition of the exchange reaction; control experiments established that cyanide was not acting as an uncoupling agent. ATP-ase activity[8] did not change as a function of oxidation–reduction state; less than 5% of the ATP added underwent hydrolysis.

TABLE I

Expt.	Carrier state	Additions	P_i^{32} incorporated mμmoles
1	Oxidized	...	43.2
	Oxidized	10^{-4}M DNP	<0.01
	Oxidized	ADP	<0.06
	Reduced	...	9.40
	Reduced	ADP	4.02
2	Oxidized	...	32.6
	Reduced	...	8.1
3	Oxidized	...	1.27
	Reduced	...	0.10
4	Oxidized	...	20.2
	Reduced	...	1.3

All tubes contained 0.006 *M* ATP (replaced by ADP as indicated below), 0.0001 *M* P_i labeled with P^{32}, and digitonin enzyme complex in total volume of 2.0 ml.; *p*H was 6.7. In Exp. 1–4 "oxidized" systems were equilibrated with air; in Exp. 1–3 "reduced" systems contained 0.01 *M* β-hydroxybutyrate in addition to above components and the reactions were carried out anaerobically. In Expt. 4 anaerobiosis was replaced by 0.001 *M* NaCN. Reactions were started by addition of ATP and P_i^{32} and run 10–20 minutes at 25°. Incorporation data were corrected for specific activity changes. Total enzyme N added was 38.0γ in Expt. 1, 36.6γ in Expt. 2, 10.7γ in Expt. 3, and 50.0γ in Expt. 4.

These findings are consistent with a hypothesis proposed earlier,[2,3] in which reduced carrier AH_2 first reacts with a factor C to form a "low-energy" complex AH_2—C. This complex is then postulated to undergo oxidation by the next carrier B to form reduced carrier BH_2 and the "high energy" carrier complex $A_{ox} \sim C$, which then is suggested to undergo phosphorolysis followed by transfer of the ~ P so formed to ADP. The experimental findings on the ATP-P_i^{32} exchange reaction reported here are consistent with this formulation but are not consistent with the hypothesis that a complex of the reduced form[5] of the carrier is the ultimate donor of "high-energy" phosphate.

Boyer, *et al.*, have reported that the ATP-P_i^{32} exchange in intact mitochondria is not significantly altered by the presence of cyanide[9]; under the same experimental conditions we can confirm this observation. However with lower concentrations of mitochondria and with special precautions to insure complete reduction of the carriers in the presence of β-hydroxybutyrate and cyanide, prior to addition of ATP and P_i^{32}, the inhibition of the exchange reaction is nearly complete.

(9) P. D. Boyer, W. W. Luchsinger and A. B. Falcone, *J. Biol. Chem.*, **223**, 405 (1956).

This investigation was supported by the U.S. Public Health Service, the National Science Foundation, and the Nutrition Foundation, Inc. A U.S. Public Health Service Postdoctoral Fellowship (C.L.W.) is gratefully acknowledged.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE
BALTIMORE 5, MARYLAND
CHARLES L. WADKINS
ALBERT L. LEHNINGER

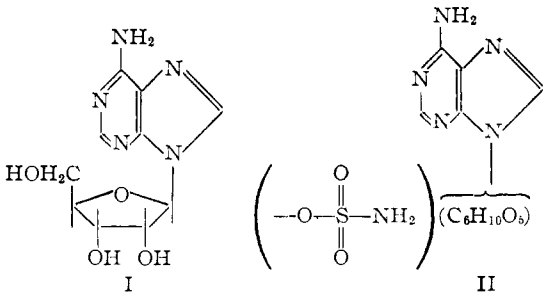
RECEIVED JANUARY 2, 1957

THE STRUCTURE OF NUCLEOCIDIN. I.

Sir:

The isolation,[1a] physical properties,[1a] and phenomenal anti-trypanosomal activity[1b] of nucleocidin have been reported recently. We now describe structural studies which show that nucleocidin is a glycoside of adenine in which sulfamic acid is bound to the carbohydrate moiety as an ester.

When hydrolyzed with ethanolic hydrochloric acid[2] the antibiotic yielded adenine, which was characterized as its sulfate and picrate. The ultraviolet spectrum of nucleocidin is nearly superimposable on the spectrum of adenosine (I) in either acid or alkali; since the ultraviolet spectra of adenine derivatives vary according to the location



I II

of substituents on the purine skeleton,[3,4] nucleocidin must be presumed to be a 9-adenyl compound.

The conditions required for acid hydrolysis, the high proportion of oxygen in the molecule, and analogy to other "nucleoside-type" antibiotics[2,5,6] all suggested that nucleocidin was a glycoside of adenine. As expected, the antibiotic yielded a reducing sugar after acid hydrolysis: the hydrolyzate gave a positive Fehling test, a precipitate with phenylhydrazine, and a spot test color with aniline phthalate.

Hydrolysis (100°, 30 min.) of the antibiotic with 2*N* hydrochloric acid containing barium chloride produced barium sulfate. The sulfur atom in nucleocidin is, therefore, hexavalent, and not linked directly to carbon, since sulfones and alkane sulfonic acids are stable under these conditions.

After hydrolysis of nucleocidin using Dowex-

(1) (a) S. O. Thomas, V. L. Singleton, J. A. Lowery, R. W. Sharpe, L. M. Pruess, J. N. Porter, J. H. Mowat and N. Bohonos, *Antibiotics Annual*, 1956–1957, in press. (b) R. I. Hewitt, A. R. Gumble, L. H. Taylor, W. S. Wallace, *ibid.*, in press. These two papers are the texts of reports read before the Fourth Annual Antibiotic Symposium, Washington, D. C., October 17–19, 1956.

(2) *Cf.* C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953).

(3) J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, 765 (1936).

(4) J. M. Gulland, R. F. Story, *ibid.*, 259 (1938).

(5) H. R. Bentley, *et al.*, *ibid.*, 2303 (1951).

(6) N. Löfgren, B. Lüning and H. Hedström, *Acta Chem. Scand.*, **8**, 670 (1954).