It is of interest that two distinct and metabolically separated steps of a biosynthetic sequence are catalyzed by one enzyme and are under the control of a common genetic unit.

(7) Predoctoral Fellow of the National Science Foundation.

DIVISION OF BIOCHEMISTRY RICHARD W. MILLER[7]
DEPARTMENT OF BIOLOGY LEWIS N. LUKENS[7]
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS JOHN M. BUCHANAN

RECEIVED FEBRUARY 16, 1957

SYNTHESIS OF DIPHOSPHOPYRIDINE NUCLEOTIDE FROM NICOTINIC ACID BY HUMAN ERYTHROCYTES *IN VITRO*[1]

Sir:

In 1943,[2] *in vitro* synthesis of pyridine nucleotides from nicotinic acid (NA)[3] by human erythrocytes was demonstrated under conditions wherein no comparable synthesis was obtained with nicotinamide (NAm). Erythrocytes were shown to be freely permeable to both compounds; however, the microbiological assay employed was non-specific and the synthesized material might equally well have been nicotinamide mononucleotide (NMN), DPN, TPN, or nicotinamide riboside. Later it was observed that, in the presence of very high concentrations of NAm, pyridine nucleotides were synthesized of which 75–95% was NMN and the remainder DPN.[4] Preiss and Handler[5] have shown NMN formation in this system to occur by condensation of NAm with 1-pyrophosphoryl ribose-5-phosphate. However, since extremely high and non-physiological concentrations of NAm were required for this reaction and since no DPN-pyrophosphorylase has been detected in human erythrocytes,[5,6] NMN may not be an intermediate in DPN synthesis in the human erythrocyte. In consequence, it appeared desirable to reinvestigate the reported synthesis of pyridine nucleotides from NA by erythrocytes and establish the nature of the synthesized material.

Table I shows that at low concentration of NA there was appreciable synthesis of pyridine nucleotide, all of which was accounted for as DPN by the alcohol dehydrogenase assay, whereas NAm at similar concentration did not elevate the pyridine nucleotide level significantly. Only at higher concentrations was NAm an effective precursor for DPN synthesis. At a concentration sufficiently great to permit significant DPN synthesis, NMN accumulated in almost equal quantity, while at still higher NAm concentration, NMN synthesis was dominant. In contrast, NMN synthesis has not been observed at any concentration of NA. Indeed, in most experiments in which DPN synthesis was observed from NA, the NMN of the erythrocyte, which usually accounts for about 50% of the total pyridine nucleotides, disappeared. With both substrates, virtually all of the total nucleotide synthesized, as measured by the fluorimetric assay, as accountable as NMN and/or DPN.

(1) These studies were supported in part by contract AT-(40-1)-289 between Duke University and the United States Atomic Energy Commission and by Grant RG-91 from the National Institutes of Health.

(2) P. Handler and H. I. Kohn, *J. Biol. Chem.*, **150**, 447 (1943).

(3) These abbreviations are used: NA, nicotinic acid; NAm, nicotinamide; GAm, glutamine; NMN, nicotinamide mononucleotide; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; TRIS, trishydroxymethylaminomethane.

(4) I. G. Leder and P. Handler, *J. Biol. Chem.*, **189**, 889 (1951).

(5) J. Preiss and P. Handler, Abstracts of the 130th National Meeting of the American Chemical Society (1956), p. 44c; *J. Biol. Chem.*, in press.

(6) A. Malkin and O. F. Denstedt, *Canadian J. Biochem. Physiol.*, **34**, 121 (1956).

TABLE I

PYRIDINE NUCLEOTIDE FORMATION FROM NICOTINIC ACID AND NICOTINAMIDE BY HUMAN ERYTHROCYTES

The reaction mixture contained: 50 μmoles phosphate pH 7.4, 22.5 mg. glucose, defibrinated whole blood 3.0 ml., NA, NAm, and glutamine in the amounts indicated. Total volume was 3.8 ml.; incubation time 22 hours.

Additions μmoles		μmoles	ΔTotal Pyridine nucleotide[a] μmoles	ΔDPN[b] μmoles	ΔNMN[c] μmoles
NA	0.3		0.045	0.041	...
NA	0.3	GAm 20	.201	.211	...
NA	1.0		.060	.049	...
NA	1.0	GAm 20	.222	.195	...
NA	10.0		.061	.059	...
NA	10.0	GAm 20	.126	.122	...
NA	100.0		.093	.139	...
NAm	0.3		.016	.000	...
NAm	1.3	GAm 20	.015	.000	...
NAm	1.0		.019	.000	...
NAm	1.0	GAm 20	.000	.000	...
NAm	10.0		.111	.048	...
NAm	10.0	GAm 20	.068	.036	...
NAm	100.0	GAm 20	.587	.323	0.208
NAm	300.0		1.15	.388	.610

[a] Systems lacking NAm and NA contained 0.193 μmole. This value was subtracted from the observed value, yielding the increment shown. [b] Increment over the control value of 0.089 μmole. [c] Assayed with alcohol dehydrogenase after aliquot was treated with DPN pyrophosphorylase and ATP. NMN was calculated as the increment in DPN due to this treatment. The control contained 0.093 μmole NMN.

It is evident from these data that free NAm cannot be an intermediate in DPN synthesis from NA, suggesting that amidation may occur after nicotinic acid is converted to some presently unknown nucleotide derivative. Several explanations might be offered to account for DPN synthesis at high NAm concentration, but further work is necessary to establish the mechanism of this process.

Table II shows that DPN synthesis from NA is dependent on phosphate, glucose, and ammonia which may be supplied as glutamine. Asparagine

TABLE II

REQUIREMENTS FOR DPN SYNTHESIS BY ERYTHROCYTES

The complete reaction mixture contained 10 μmoles NA, 10 μmoles GAm, 50 μmoles phosphate pH 7.4, 22.5 mg. glucose, 20 μmoles Mg^{++}, defibrinated blood 3.0 ml., 0.9% NaCl to 4.74 ml., incubation time 21 hours.

Omissions	Final DPN, μmole	ΔDPN, μmole
None	0.285	0.148
NA	.137	.000
TRIS instead of phosphate	.153	.016
Glucose	.180	.043
Mg^{++}	.294	.157
GAm	.182	.045
NH_4^+ instead of GAm	.276	.139
Asparagine instead of GAm	.199	.062

showed almost no activity as the amide donor. Under the conditions of the experiment shown in Table II, glutamine supply limits DPN synthesis. Thus, in a separate experiment under similar conditions, with NA held constant at 10 μmoles per vessel, DPN synthesis in the presence of 0, 4, 10 and 20 μmoles of glutamine was 0.052, 0.104, 0.172 and 0.274 μmole, respectively. Further investigations are in progress seeking to elucidate the mechanism of pyridine nucleotide synthesis from nicotinic acid and its amide.

(7) Predoctoral Fellow of the National Institutes of Health.

DEPARTMENT OF BIOCHEMISTRY
DUKE UNIVERSITY SCHOOL OF MEDICINE J. PREISS[7]
DURHAM, NORTH CAROLINA PHILIP HANDLER

RECEIVED FEBRUARY 11, 1957

THE ISOTROPIC LENGTH OF POLYMER NETWORKS

Sir:

A general theory of the elastic properties of polymer networks was developed in a recent paper[1] and this theory was applied to the cross-linking of highly oriented chains. Whereas for a network formed in the usual way by cross-linking chain molecules in random arrangement the isotropic length L_i of the network (*i.e.*, its length under no stress) must obviously be independent of the degree of cross-linking, it was shown that for a network formed by the random cross-linking of highly oriented chains L_i should increase directly as the square root of the fraction ρ of the units cross-linked. Although it has been reported that the cross linking of stretched rubber results in an increase in its isotropic (zero stress) length,[2,3] adequate data are not available to test the aforementioned deduction. We wish to report the results of studies of the isotropic length of natural rubber networks formed from chains in a highly oriented state. These results give strong support to the theoretical conclusions.

The highly oriented state of the rubber, prior to cross-linking is obtained by modification of the "racking process" originally described by Feuchter.[4] The wide angle X-ray pattern[5] indicates that the specimen is in a highly oriented state and the ratio of the extended length to retracted length is about eleven. The samples were cross-linked by subjecting them to γ-ray irradiation from a Co^{60} source. The efficiency of cross-linking in the highly oriented racked rubber was found to be twice that for unoriented rubber.

In Fig. 1 the ratio of L_i to the initial length L_0 is plotted against $\rho^{1/2}$. A fiftyfold range in cross-linking is encompassed by these experiments and the isotropic length increases by a factor of two and a half. At the higher degrees of cross-linking the data are well represented by a straight line which extrapolates to the origin. However, as the cross-

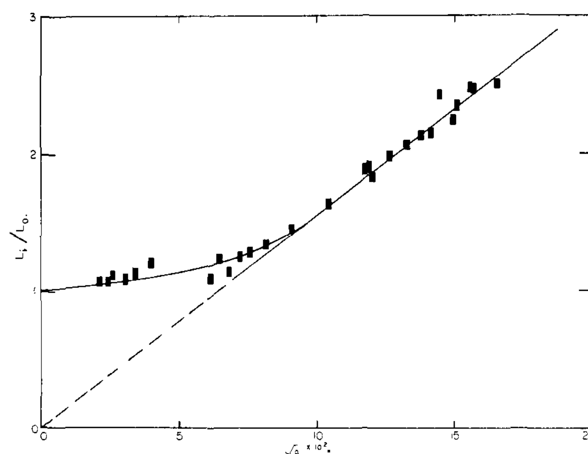

Fig. 1.—Plot of ratio of isotropic length after cross-linking L_i to initial length L_0 against the square root of the fraction of the units crosslinked $\rho^{1/2}$.

linking density decreases deviations from linearity occur and L_i/L_0 appears to approach unity. According to equation (38) of ref. 1, L_i/L_0 should vary directly as $\rho^{1/2}$ for chains with *perfect* axial orientation, and for an infinitesimal amount of cross-linking L_i should shrink to zero. This behavior is indicated by the linear portion of the curve and its extrapolation to the origin. Since the chains prior to network formation are neither completely nor perfectly oriented, deviations from linearity would be expected at low cross-linking densities where L_i should tend to remain constant as observed. The slope of the linear portion of the curve is fifteen while theoretically it is estimated to be about ten. It appears that "racked rubber" can serve as a good model for the physical behavior of the fibrous proteins.

Further details of the experimental methods, a more thorough discussion of these results as well as a comparison of the isotropic melting temperature and swelling behavior of different type networks will appear in a forthcoming paper.[6]

(1) P. J. Flory, THIS JOURNAL, **78**, 5222 (1956).
(2) R. D. Andrews, E. E. Hanson and A. V. Tobolsky, *J. Appl. Phys.*, **17**, 352 (1946).
(3) J. P. Berry, J. Scanlan and W. F. Watson, *Trans. Faraday Soc.* **52**, 1137 (1956).
(4) H. Feuchter, *Kautschuk*, Dec., p. 6 (1925); pp. 8, 28 (1928).
(5) C. C. Davis and J. T. Blake, "The Chemistry and Technology of Rubber," Reinhold Publishing Corporation, New York, N. Y., 1937, p. 78.
(6) D. E. Roberts and L. Mandelkern, in preparation.

NATIONAL BUREAU OF STANDARDS DONALD E. ROBERTS
WASHINGTON 25, D. C. LEO MANDELKERN
BAKER LABORATORY OF CHEMISTRY
CORNELL UNIVERSITY PAUL J. FLORY
ITHACA, N. Y.

RECEIVED JANUARY 28, 1957

ADRENAL HORMONES AND RELATED COMPOUNDS. V. FLUORINATED 6-METHYL STEROIDS

Sir:

We recently have reported[1] the preparation of a number of 6-methylated analogs of adrenal hormones which show unusual potentiation of glucocorticoid activity with no sodium-retaining properties. The group of 9α-fluoro- and 21-fluoro-6-methyl steroids reported herein represents a continuation of this work. Compound III described below is by far the most potent glucocorticoid reported to date.

(1) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek and J. A. Hogg, THIS JOURNAL, **78**, 6213 (1956).