solvent containing 80% *n*-propanol and 20% water. Two bands were observed under an ultraviolet lamp. Their *Rf*'s, 0.54 and 0.39, correspond with those of 4-amino-5-imidazolecarboxamide and its riboside, respectively.[8] Ribose and ribose phosphate do not migrate from the origin in this solvent. The ultraviolet absorption curve of the riboside had a maximum at 267 mμ. Analysis of the eluted compound by ultraviolet absorption (using $E_{267\ m\mu} = 1.27 \times 10^4$), diazotizable amine,[9] and the orcinol reaction[10] gave a 1:1:1 ratio. Approximately 0.08 μM of the riboside was recovered from the paper chromatogram. When either D-ribose or ribose-5-phosphate was substituted for ribose-1-phosphate in the incubation medium only the 4-amino-5-imidazolecarboxamide band appeared.

The 4-amino-5-imidazolecarboxamide riboside isolated in these experiments has the same properties as that isolated by Greenberg[8] from a culture of *E. coli* whose growth was inhibited by sulfonamides. The synthesis of 4-amino-5-imidazolecarboxamide deoxyriboside has been demonstrated in suspensions of *E. coli* B,[11] and by a *trans*-N-glycosidase reaction in an enzyme preparation from *L. helveticus*.[12]

Greenberg[13] has reported that 4-amino-5-imidazolecarboxamide riboside may be converted to its ribotide by the action of adenosine triphosphate in the presence of pigeon liver extract. The present experiments, when coupled with those of Greenberg provide analogy for a metabolic pathway for the conversion of the carboxamide to its ribotide and to inosinic acid in pigeon liver, a series of reactions which were previously postulated on the basis of experiments with radioactive carboxamide[14] and experiments with inosinic acid and radioactive formate.[15]

When adenine and ribose-1-phosphate were incubated with the nucleoside phosphorylase, a rapid formation of inosine was noted. The enzyme preparation completely converted adenosine to inosine or to inosine and hypoxanthine in the presence of inorganic phosphate. As the formation of hypoxanthine from adenine (in the absence of ribose-1-phosphate) could not be detected, it seems probable that adenine and ribose-1-phosphate had reacted to form adenosine which was then deaminated to inosine. These results are in contrast to those of Kalckar,[16] who was unable to find any reaction between adenine and ribose-1-phosphate in the presence of his preparation of rat liver nucleoside phosphorylase.

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THE OCCURRENCE OF A NEW FLAVIN DINUCLEOTIDE (FAD-X)

Sir:

During a recent investigation[1,2] concerned with the isolation of flavin-adenine-dinucleotide (FAD) from animal tissues, the existence of a new flavin nucleotide, (FAD-X), not identical with any of the known flavins, was noted. Specifically, when the flavin concentrates were subjected to partition chromatography between phenol–butanol and water, or adsorption chromatography on dicalcium phosphate, FAD-X, FAD and flavin mononucleotide (FMN) were separated. Each of these flavins could be rechromatographed on either column as a single band. The relative amount of FAD-X, compared to FAD, ranged approximately from 5 to 15% for a large number of preparations.

Using paper chromatography, a comparison of FAD-X with other flavins is given in Table I. In many solvent systems FAD-X is indistinguishable from FAD; however, in systems such as 3 and 4, the two are readily separated.

Analysis of FAD-X indicates a flavin:phosphate:adenine ratio of 1:2:1. FAD-X is similar to FAD in several other respects: absorption spectrum, dependence of fluorescence upon *p*H[3] and the formation of insoluble complexes with heavy metals. It has no coenzyme activity, however, with the D-amino acid apo-oxidase.[4]

Enzymatic cleavage of FAD-X with nucleotide pyrophosphatase,[5] kindly supplied by Dr. A. Kornberg, yields a flavin mononucleotide FMN-X and adenosine-5′-phosphate (AMP); upon acid hydrolysis a mixture of FMN and FMN-X are produced. FMN and FMN-X may be separated by paper chromatography, as shown in Table I. As judged by paper chromatography, FMN-X is identical with *cyclic* riboflavin-4′,5′-phosphate (cyc-FMN), kindly supplied by Dr. A. R. Todd.[6] Exposed to ammonia, FAD is converted to cyc-

TABLE I

R_f VALUES FOR ASCENDING PAPER CHROMATOGRAMS[a]

Col. (1) 5% Na_2HPO_4 in H_2O; (2) 4/1/5 *n*-butanol/acetic acid/water (top phase); (3) 160 g. phenol/30 ml. *n*-butanol/100 ml. water (lower phase); (4) collidine saturated with water.

	1	2	3	4
FAD	0.40	0.05	0.23	0.17
FAD-X	.40	.05	.47	.30
FMN	.54	.10	.17	.04
FMN-X	.54	.13	.50	.15
Rb	.30	.30	.79	.69
Lyxoflavin[b]	.29	.32	.77	.66
Riboflavinyl glucoside[c]	.40	.22	.60	.50
Lumichrome	.07	.68	.88	.72
Lumiflavin	.18	.48	.94	.68

[a] Whatman No. 1 paper. [b] Kindly supplied by Dr. Karl Folkers. [c] Kindly supplied by Dr. L. G. Whitby.

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FMN.[6] FAD-X is an intermediate in this process and under relatively mild conditions, such as exposure of FAD spots to ammonia vapor before paper chromatography, a quantitative conversion to FAD-X may be effected.

Treatment of FMN-X with intestinal phosphatase (Armour) yields a product which is chromatographically identical with riboflavin, thus excluding riboflavinyl glucoside.[7] Microbiological assays,[8] kindly performed by Dr. E. E. Snell, have shown that FAD-X contains riboflavin and not lyxoflavin.[9,10]

The above evidence supports the hypothesis that FAD-X is a flavin dinucleotide, isomeric with FAD, but having a *cyclic* phosphate structure. It cannot be stated at present whether FAD-X occurs naturally or is produced artificially during the isolation of FAD.

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THE MECHANISM OF THE REACTION BETWEEN OZONE AND NITROSYL CHLORIDE

Sir:

Schumacher and Sprenger[1] produced nitryl chloride by the reaction of ozone with nitrosyl chloride which they described as "rapid and complete"

$$NOCl + O_3 = NO_2Cl + O_2 \qquad (1)$$

This reaction is an interesting analog of the reaction between nitrogen dioxide and ozone,[2] and also it has an additional feature of interest in that it involves a forbidden electronic transition.[3] By following nitrosyl chloride by its absorption of the 405 mμ Hg line, we have followed this "rapid" reaction in a meter-long Pyrex tube by the same method used with nitrogen dioxide and ozone.[2] Our preliminary results were erratic, irreproducible, and partially heterogeneous. There was an induction period, followed by an increase in rate which went through a maximum and fell to zero as the reactants were consumed. We went to great lengths to remove all impurities from the reactants and used a cell with smaller surface to volume ratio. Under these conditions the reaction appeared to cease altogether. We finally concluded that the reaction as written above does not occur at all.

Ozone produced by an electric discharge contains traces of nitrogen pentoxide if the oxygen stream contains traces of nitrogen. Also nitrosyl chloride could be contaminated with nitric oxide or nitrogen dioxide, and ozone would rapidly convert either of these to nitrogen pentoxide. Therefore we deliberately added small amounts of nitrogen pentoxide to the ozone stream to examine the effect of a probable impurity. A fast homogeneous reaction set in with the stoichiometry of Eq. 1. With a large excess of ozone, the reaction in the presence of nitrogen pentoxide was found to be one-half order in nitrosyl chloride. By correcting for this dependence on nitrosyl chloride, rates were found to be one-half order in ozone, and all runs from start to finish were one-half order in each of the two reactants. When the log of these rate constants was plotted against the log of the catalyst concentration, the slope was very nearly one-half, indicating one-half order dependence on nitrogen pentoxide. Thus the empirical rate expression was

$$-d[NOCl]/dt = k[NOCl]^{1/2}[O_3]^{1/2}[N_2O_5]^{1/2} \qquad (2)$$

The following mechanism is proposed for this reaction

$$N_2O_5 \xrightarrow{k_1} NO_2 + NO_3$$

$$NO_2 + NO_3 \xrightarrow{k_2} N_2O_5$$

$$NO_2 + O_3 \xrightarrow{k_3} NO_3 + O_2$$

$$NOCl + NO_3 \xrightarrow{k_4} NO_2Cl + NO_2$$

By making the steady-state assumption for nitrogen dioxide and NO_3, the rate expression exactly derived from the mechanism is

$$\frac{-d[NOCl]}{dt} = \left(\frac{k_1k_3k_4}{k_2}\right)^{1/2}[NOCl]^{1/2}[O_3]^{1/2}[N_2O_5]^{1/2} \qquad (3)$$

which agrees with the observed rate function. The values of k_1/k_2 and k_3 are known,[4] and by substituting the values and the observed rate into Eq. (3) we find k_4 to be 0.7×10^8 cc. mole^{-1}sec.$^{-1}$ at 40°. If the pre-exponential factor is about 10^{12} cc. mole^{-1}sec.$^{-1}$, the energy of activation of k_4 is about 6 kcal.

It is interesting to notice that while Eq. (1) involves a forbidden electronic transition, no step in the proposed mechanism is forbidden. In the work of Schumacher and Sprenger there must certainly have been some unsuspected nitrogen pentoxide present. With initially pure reactants our work indicated heterogeneous catalysis for the equilibrium $2NOCl \rightleftarrows 2NO + Cl_2$ followed by rapid reaction of nitric oxide and ozone to produce nitrogen pentoxide, which then catalyzes reaction (1).

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