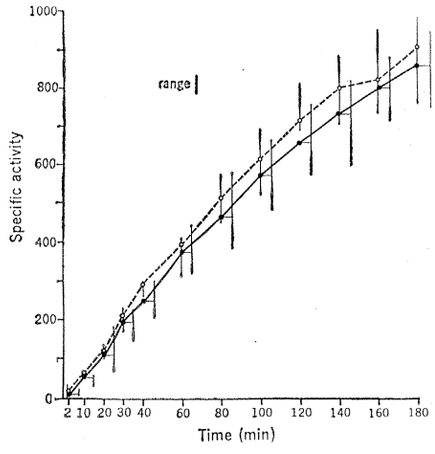

Fig. 1. Output of $C^{14}O_2$ after intake of ethanol-1-C^{14} by alcoholic and nonalcoholic subjects. Specific activity is expressed in picocuries per millimole of CO_2 per liter. Dashed line, six alcoholics; solid line, six nonalcoholics.

being 0.52 g of ethanol per kilogram of body weight. This dose contained 0.024 μc of ethanol-1-C^{14} per gram of nonradioactive ethanol (*11*). Each subject drank his ethanol solution within a 2-minute period of time. Peak concentrations of ethanol in the blood (between 50 and 60 mg per 100 ml) occurred 20 minutes after ingestion for both alcoholic and nonalcoholic subjects. This approximates the optimum ethanol concentration for maximum activity of alcohol dehydrogenase in the human liver (*12*).

After ingestion of ethanol at zero time, expired breath samples were collected at 2, 10, 20, 30, 40, 60, 80, 120, 140, 160, and 180 minutes. Carbon dioxide in breath samples was absorbed in 2*N* sodium hydroxide. Carbon dioxide was regenerated by acidification with concentrated sulfuric acid in a vacuum line apparatus to remove water through differential cooling with liquid nitrogen and dry ice in acetone. Gaseous carbon dioxide was determined manometrically in this apparatus, and a fraction of the CO_2 samples was absorbed in redistilled liquid Primene (*11*).

The counting of $C^{14}O_2$ was carried out in a scintillation-fluid mixture of 0.6 percent 2,5-diphenyloxazole and 0.0075 percent 1,4-bis[2-(5-phenyloxazolyl)]-benzene in toluene in a Packard Tri-Carb scintillation spectrometer with a C^{14}-counting efficiency of 77 percent. No significant quenching occurred as assessed by both internal-standard and channels-ratio methods.

The $C^{14}O_2$ was detected in expired breath samples 2 minutes after ethanol ingestion (Fig. 1). Thereafter, $C^{14}O_2$ increased linearly up to and including 100 minutes. From 100 to 180 minutes, output of $C^{14}O_2$ was curvilinear. This portion of the curve corresponded with concentrations of 20 mg or less of alcohol per 100 ml of blood.

The data indicate that there are no significant differences in the rates of ethanol metabolism in alcoholic and nonalcoholic individuals after administration of a large dose of alcohol. This finding is consistent with those of other investigators who have been unable to find differences in rates of ethanol metabolism in alcoholics as contrasted with those in normal subjects. The enhancement of ethanol metabolism after chronic ingestion of ethanol observed in alcoholics and nonalcoholics (*4*) is not manifested when subjects are abstinent for a period of 3 weeks. It is likely that any enzymatic induction produced by prolonged ethanol ingestion is transitory and does not persist when an individual ceases drinking.

These data do not preclude the existence of a unique pathway for ethanol metabolism in alcoholics. New intermediates in the metabolism of ethanol (*13*) for rat tissue may exist in man; also, even small amounts of such intermediates may have significant pharmacological or pathological effects. However, it is unlikely that any large quantities of new or already identified intermediates accumulate or are retained in alcoholics as contrasted to nonalcoholics. My findings lend support to the conclusions of Westerfeld and Schulman (*8*) that tolerance to alcohol is related to processes of adaptation in the central nervous system rather than to alterations in the rate of metabolism of ethanol.

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13 November 1967

Binuclear Ion Containing Nitrogen as a Bridging Group

Abstract. *A binuclear ion* $[(NH_3)_5RuN_2Ru(NH_3)_5]^{4+}$ *is formed by the direct reaction of* N_2 *with an aqueous solution of* $(NH_3)_5RuOH_2^{2+}$ *at room temperature. The binuclear ion is also formed by the reversible reaction of* $(NH_3)_5RuOH_2^{2+}$ *with* $(NH_3)_5RuN_2^{2+}$. *Solid* $[(NH_3)_5RuN_2Ru(NH_3)_5]$ $(BF_4)_4$ *has been prepared, and its ultraviolet and infrared spectra are reported.*

The discovery by Allen and Senoff (*1*) of the ion $(NH_3)_5RuN_2^{2+}$ is important in showing that a combination of metal ion and N_2 once formed can persist. Of equal significance for the fixation of nitrogen by homogeneous catalysis in solution is Allen and Senoff's (*1*) discovery that coordinated nitrogen is much more readily reduced than free nitrogen is.

The observation (*2*) that $(NH_3)_5RuOH_2^{2+}$ (*3*) reacts spontaneously in aqueous solution with N_2 to form Allen and Senoff's ion demonstrates a remarkable thermodynamic stability for this nitrogen complex and, furthermore, shows that the capacity of a metal ion to combine with N_2 can exceed its capacity to reduce H^+. The fact that the equilibrium constant for the reaction

$$H_2 + 2(NH_3)_5RuOH_2^{3+} = 2H^+ + 2(NH_3)_5RuOH_2^{2+}$$

is greater than 10^3 (*4*) shows that $(NH_3)_5RuOH_2^{2+}$ is a weaker reducing agent than H_2, so that even when H^+ is at unit activity, with H_2 at 1 atm,

Ru(III) will be largely reduced to Ru(II) when equilibrium is reached. In retrospect, the fact that N_2 can react with a metal ion which is not powerful enough to reduce H_2O (or even H^+) is not surprising, if N_2, in forming complexes, is accepted as being analogous to CO. The total requirement for stable complex formation can be quite different from that for net oxidation of the metal ion.

The mechanism of reduction of molecular N_2 to NH_3 by nitrogen-fixing bacteria is an important biochemical problem. Molecular N_2 may form a complex with a metal ion, initiating the reduction in biological systems. To reduce N_2 complexed to a metal ion, unless the metal readily undergoes oxidation by many electrons, additional reducing equivalents are required. Additional metal ions can supply the needed reducing equivalents, and binuclear complexes have been suggested as intermediates in symbiotic nitrogen fixation (*5*).

We show here that the new species absorbing at 262 mμ, which forms (*2*) in addition to Allen and Senoff's ion when N_2 and $(NH_3)_5RuOH_2^{2+}$ are brought together, is the binuclear ion

$$[(NH_3)_5RuN_2Ru(NH_3)_5]^{4+}$$

This ion can be formed by the interaction of $(NH_3)_5RuN_2^{2+}$ with $(NH_3)_5RuOH_2^{2+}$ as follows.

When $(NH_3)_5RuOH_2^{2+}$ and $(NH_3)_5RuN_2^{2+}$ are mixed in solution, the absorption at 221 mμ characteristic of the latter ion (*2*) slowly diminishes, while at the same time the absorption at 262 mμ increases. The stoichiometry of this reaction was investigated with the use of Job's method (*6*). A solution containing $(NH_3)_5RuOH_2^{2+}$ was prepared by reducing $(NH_3)_5RuCl_3$ in 0.1*M* H_2SO_4 with amalgamated Zn, and a solution containing $(NH_3)_5RuN_2^{2+}$ was prepared from $(NH_3)_5RuN_2Cl_2$. Several solutions were made up from these stock reagents with varying initial ratios of $(NH_3)_5RuN_2^{2+}$ to $(NH_3)_5RuOH_2^{2+}$ but with 0.030*M* $(NH_3)_5Ru(II)$ as the total concentration. After several hours, portions were removed and diluted, and the absorption spectra were recorded. The results show that $(NH_3)_5RuN_2^{2+}$ and $(NH_3)_5RuOH_2^{2+}$ react with 1 : 1 stoichiometry to form the new species (Fig. 1).

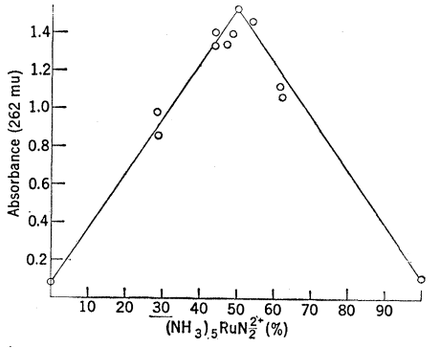

Fig. 1. Relative absorbances at 262 mμ of isomolar solutions with varying ratios of $[(NH_3)_5RuN_2^{2+}]_0/[(NH_3)_5RuOH_2^{2+}]_0$. The $[(NH_3)_5Ru(II)]_0$ in the reaction mixture was 0.030*M*; after dilution to measure absorbance, it was about $7 \times 10^{-5}M$. The fact that the absorbance is at a peak at 50 percent $(NH_3)_5RuN_2^{2+}$ shows that the stoichiometry is 1 : 1; the fact that the maximum is sharp shows that the reaction is essentially complete.

Two lines of evidence based on solution chemistry show that the reaction between $(NH_3)_5RuN_2^{2+}$ and $(NH_3)_5RuOH_2^{2+}$ involves addition (Q is the addition product of unknown structure):

$$(NH_3)_5RuN_2^{2+} + (NH_3)_5RuOH_2^{2+} = Q + H_2O \quad (1)$$

rather than the redox process [Q′ is the redox product derived from $(NH_3)_5RuN_2^{2+}$]:

$$(NH_3)_5RuN_2^{2+} + (NH_3)_5RuOH_2^{2+} = Q' + (NH_3)_5RuOH_2^{3+} \quad (2)$$

No evidence for the formation of $(NH_3)_5RuOH_2^{3+}$ by the reaction in question is found even when Cl^- is present. Chloride ion would react with $(NH_3)_5RuOH_2^{3+}$ to form $(NH_3)_5RuCl^{2+}$ which absorbs at 327 mμ; however, no such absorption is observed. Second, it is found that the new species transforms to Allen and Senoff's ion on dilution. Figure 2 shows successive traces of the absorption spectrum of a solution at about *p*H 10.4, which initially contained a relatively high proportion of the new species. As time progresses, the absorption at 221 mμ increases, while that at 262 mμ decreases. Over a considerable time span, the system is well behaved, all the curves passing through an isosbestic point. The affinity of $(NH_3)_5RuOH_2^{2+}$ for $(NH_3)_5RuN_2^{2+}$ is quite high, and even at $10^{-5}M$ the dissociation of the binuclear ion, according to the reverse of reaction 1, is only about 60 percent complete at equilibrium. At ordinary concentrations of $(NH_3)_5RuOH_2^{2+}$, the binuclear ion rather than that of Allen and Senoff is the dominant product of the reaction of N_2 with $(NH_3)_5RuOH_2^{2+}$.

Taking advantage of this observation, we have prepared crystalline solids containing the new species by the following procedure. A solution 0.05*M* in $(NH_3)_5RuCl_3$ and 0.1*M* in H_2SO_4 was reduced by amalgamated Zn, under helium. After 50 minutes the solution, which was no longer acid to *p*H paper, was separated from metallic zinc, and gaseous nitrogen was bubbled into it for 10 hours. At the end of this time the solution was filtered, still in the absence of air, into a filtered solution of concentrated anion (NaBr and HBr or $NaBF_4$ and dilute H_2SO_4). The solid that formed was separated, washed with acetone and ether, and then air-dried.

Partial elementary analysis of presumed $[(NH_3)_5Ru]_2N_2(BF_4)_4$ shows that H was 4.2 to 4.3 percent, N was 21.06 percent, and B was 5.61 percent, compared to the theoretical values of 4.04, 22.5, and 5.79 percent, respectively. More significant for the N_2 content of the complex than the elementary analysis for nitrogen (*7*) is the amount of N_2 released on oxidation by

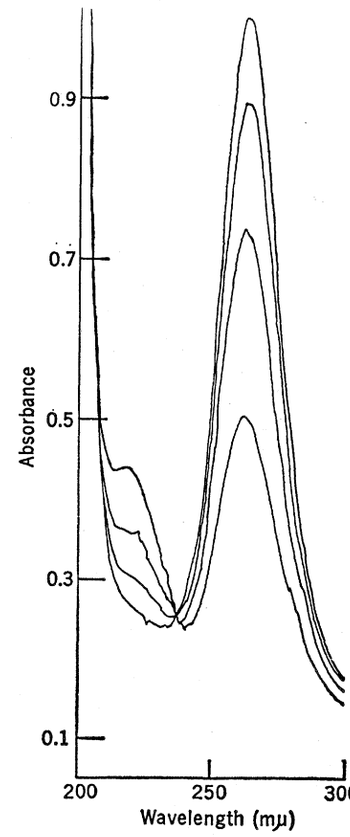

Fig. 2. Decomposition of $[(NH_3)_5Ru]_2N_2^{4+}$ (maximum wavelength, 262 mμ) leading to $(NH_3)_5RuN_2^{2+}$ (maximum wavelength, 221 mμ). In descending order of absorbance at 262 mμ the reaction times are 32 seconds, 2 minutes 32 seconds, 7 minutes 35 seconds, and 38 minutes 35 seconds.

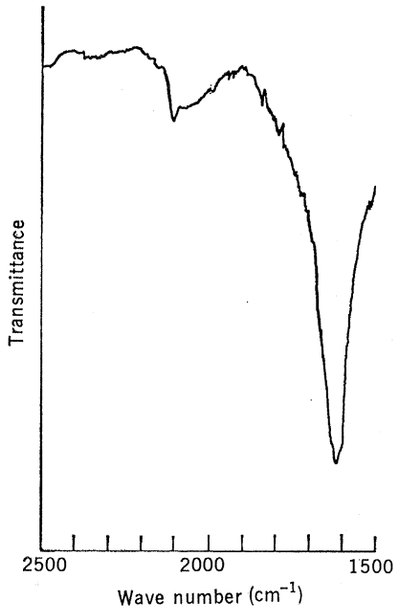

Fig. 3. Infrared spectrum of $[(NH_3)_5Ru]_2N_2$ $(BF_4)_4$.

Ce(IV). The $(NH_3)_5Ru$ moiety loses its capacity to retain N_2 when it is oxidized above the +2 state; Ce(IV) brings about this oxidation without producing N_2 from coordinated NH_3 or from NH_4^+ (*8*). When about 8 moles of Ce(IV) per mole of the new species was used the gas liberated on oxidation was shown to be more than 99 percent N_2, and the amount corresponded to 95 percent of that expected for the binuclear formulation after applying a correction for the N_2 released from the $(NH_3)_5RuN_2^{2+}$ present in small amount.

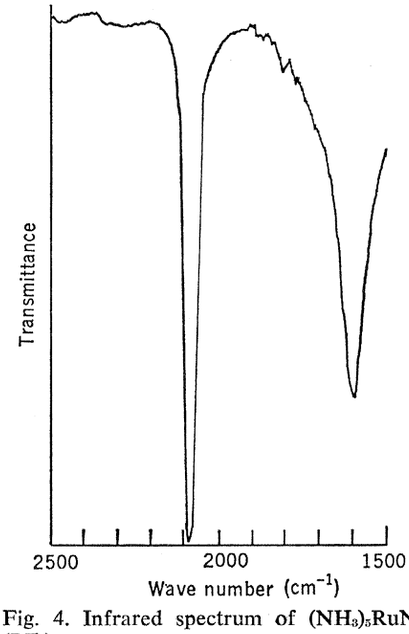

Fig. 4. Infrared spectrum of $(NH_3)_5RuN_2$ $(BF_4)_2$.

The portion of the infrared spectrum of a sample of $[(NH_3)_5Ru]_2N_2$ $(BF_4)_4$ (Fig. 3) provides an interesting comparison with the spectrum of $[NH_3)_5RuN_2]$ $(BF_4)_2$ (Fig. 4). In place of the strong, sharp peak at about 2100 cm^{-1} characteristic of the N–N stretch in $(NH_3)_5RuN_2^{2+}$, there is a broad, weak absorption at about 2060 cm^{-1} [the small spike at about 2100 cm^{-1} in Fig. 3 can be attributed to residual $(NH_3)_5RuN_2^{2+}$]. The broad absorption may arise from the N–N stretch in the binuclear species or from an entirely different vibration. In any event, the absence of a strong absorption in the spectrum attributable to the N–N stretching frequency implies that N_2 is symmetrically bound in the binuclear ion.

The extinction coefficient (ϵ) of the binuclear ion at 262 mμ, as measured for a solution prepared from the tetrafluoborate salt, is 4.7×10^4, or about 15 percent higher than the value calculated from the data shown in Fig. 1. The reason for this discrepancy is not known. A possibility is that coordinated ammonia is labilized when N_2 is complexed and that the replacement of ammonia by water has taken place to a differing extent in the two systems. The value of ϵ for $(NH_3)_5RuN_2^{2+}$ at its maximum, 221 mμ, is $1.6 \pm 0.1 \times 10^4$. [This is a correction of the value of $\epsilon = 1.3 \pm 0.1 \times 10^4$ given in the previous communication (*2*); the preparation of $[(NH_3)_5Ru]N_2$ $(BF_4)_2$ has been found to contain a considerable admixture of $(NH_3)_5Ru$(III).]

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Guinea Pig Complement: Two Active Forms of First Component

Abstract. *By solubility chromatography at pH 7.5 and low ionic strength, a partially purified preparation of the first component of guinea pig complement was separated into two fractions of different solubility under these conditions. On rechromatography, each fraction emerged in the same position as it had originally.*

In solubility chromatography, proteins are allowed to precipitate on a highly cross-linked gel column equilibrated with a solution in which the proteins of interest are insoluble. The proteins are then redissolved in an advancing front or gradient of solvent, but they precipitate again, as they migrate ahead of the solvent, as a result of the gel-filtration effect of the column, to await redissolution by the solvent front. The result is a countercurrent process from which the different proteins in a mixture emerge in positions corresponding to their solubilities under the conditions chosen (*1*). This method has been applied to purification of the first component of guinea pig complement (*C′*1), which is insoluble at *p*H 5.6 and low ionic strength (*2*); it permits isolation of *C′*1 in high yield, with at least 40-fold purification (*3*); details of this procedure will be reported elsewhere. In view of the report of Nelson *et al.* (*4*), indicating that *C′*1 also precipitates at low ionic strength at *p*H 7.5, further purification was attempted by solubility chromatography at this *p*H; thus I separated the active material into two fractions: one is soluble at an ionic strength of 0.0105, the other emerges from the column at an ionic strength of about 0.055.

Assays of *C′*1 were performed at an ionic strength of 0.0652, in the presence of sufficient D-mannitol to render the solutions isotonic, according to a modification of the procedure (*5*) already described; the modification involved 1-hour incubation of the mixture of *EAC′*4 and *C′*1 at 37°C (*6, 7*) before addition of *C′*2, followed by 30 minutes at 30°C to permit formation of *SAC′* 1*a*,4,2*a* (*7*). A 5-cm (internal diameter) column containing about 75 g of polyacrylamide gel (*8*) was equilibrated with 10 m*M* tris-HCl, *p*H 7.0 (measured at room temperature), containing 0.5 m*M* $CaCl_2$; equilibration was verified by both con-