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25 January 1965

Electron Yield in the γ-Radiolysis of Water Vapor

Abstract. *Nitrous oxide at low concentrations reduces the high yield of hydrogen from γ-irradiated water vapor containing methanol from 8.9 per 100 electron volts absorbed to a plateau value of 5.9. This indicates that both electrons (yield 3.0) and hydrogen atoms (yield 5.4) are precursors to the hydrogen, the former being scavenged by nitrous oxide.*

The high yield of hydrogen, $G(H_2)$, produced when various additives are present during the γ-irradiation of water vapor has been interpreted (*1*) in terms of reactions of H atoms:

$$H_2O \rightarrow H, OH, H_2$$
$$H + CH_3OH \rightarrow H_2 + \dot{C}H_2OH$$

These H atoms are believed to originate from both the excitation and ionization of H_2O, and for the latter the reactions

$$H_2O \rightarrow H_2O^+ + e^- \quad (1)$$
$$H_2O^+ + H_2O \rightarrow H_3O^+ + HO \quad (2)$$
$$H_3O^+ + e^- \rightarrow 2H + OH \text{ or } H_2O + H \quad (3)$$

have been suggested.

Using experimental methods described (*1*) we have made observations which support the idea that two precursors to H atoms are present. The presence of 0.1 percent of nitrous oxide is sufficient to reduce $G(H_2)$ from a mixture of water vapor and methanol from 8.9 to a plateau value of 5.9, which remains constant over a fivefold increase of N_2O (Fig. 1). Nitrogen is also produced with $G(N_2) = 3.8$.

As in the case of aqueous systems (*2*) and gaseous propane (*3*), it seems probable that N_2O acts as an electron scavenger

$$N_2O + e^- \rightarrow N_2 + O^-$$

thus preventing reaction 3. If this is so, then a yield of electrons $G(e) = 3.0$ is produced, and, since the yield of hydrogen as molecules is 0.5, this would leave an H-atom yield $G(H) = 5.4$.

If H atoms arise entirely from excitation, then the extent is about twice that of ionization. However, H atoms may originate partly from the ultimate neutralization process. The following sequence seems feasible:

$$O^- + H_2O \rightarrow OH^- + OH$$
$$H_3O^+ + OH^- \rightarrow H_2O + H + OH$$

If this does occur, then the original neutralization, reaction 3, must produce two H atoms and there are about equal amounts of excitation and ionization.

We also find that in the absence of methanol the same range of N_2O concentration gives $G(N_2) = 3.0 \pm 0.4$ (10 experiments) in water vapor. This is essentially the same as the decrease in $G(H_2)$ when methanol is present, but less than $G(N_2)$ in these conditions. The higher $G(N_2)$ probably originates from the reaction

$$N_2O + \dot{C}H_2OH \rightarrow N_2 + OH + CH_2O \quad (4)$$

In support of this we have confirmed an earlier observation (*4*) that N_2O in pure methanol vapor gives rise to a chain reaction, since values of $G(N_2)$ are as high as 50.

However, with pure methanol N_2O does not affect $G(H_2)$, even though it would appear that the charge-neutralization process analogous to that in water occurs

$$CH_3OH_2^+ + e^- \rightarrow CH_3OH + H$$

and will be prevented by N_2O. It would seem that the alternative neutralization also produces a hydrogen atom and might be

$$CH_3OH_2^+ + OH^- \rightarrow CH_3OH + H + OH$$

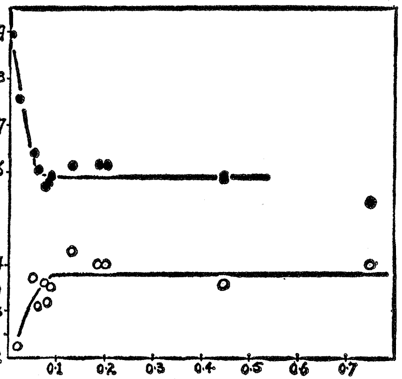

Fig. 1. Hydrogen and nitrogen yields in the γ-radiolysis of water vapor containing 2.35 moles percent methanol at 120°C. Dose: 10^{19} ev g^{-1}.

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28 January 1965

Iron Accumulation in Cockerel Plasma after Estrogen: Relation to Induced Phosphoprotein Synthesis

Abstract. *In cockerels a single injection of estrogen causes a rise of several fold in the plasma iron. At 18 hours this response is proportional to the amount of diethylstilbestrol injected and may be used as a convenient measure of estrogen action. The phenomenon is probably related to the estrogen-induced synthesis of iron-binding phosphoproteins.*

The blood of laying hens contains a phosphoprotein fraction that can also appear in cockerels treated with estrogen (*1, 2*). Most of this phosphoprotein, in both laying hens (*3*) and estrogenized cockerels (*4*), is identical to the egg protein, phosvitin. Phosvitin has a high iron-binding potential (*5*) and such a complex may account for the total iron content of egg yolk (*6*). We now report on the possible correlation between the phosphoprotein and the iron content of plasma.

White Leghorn cockerels (100 to 150 g) and 3-month-old laying hens were used. The method for the estimation of plasma iron was based on that of Tompsett (*7*). This method gave the same values for total inorganic iron as those obtained with ashed samples of hemoglobin-free material. Plasma (0.5 ml) was heated in a boiling water

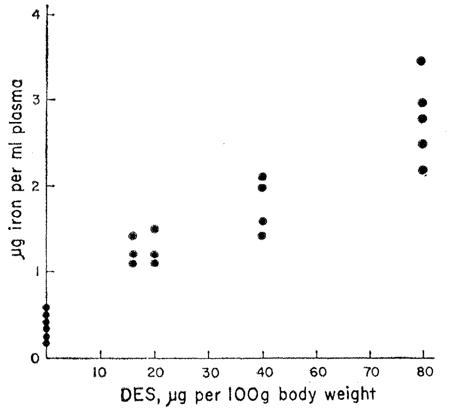

Fig. 1. The concentration of plasma iron in cockerels after the administration of varying doses of diethylstilbestrol (DES).

bath for 10 minutes and homogenized with 1.25 ml of thioglycolic acid (20 percent). After 20 minutes at room temperature, trichloroacetic acid (0.75 ml of a 30-percent solution) was added, the mixture was centrifuged, the supernatant was neutralized with concentrated ammonium hydroxide, and the intensity of the resulting purple color was measured at 520 mµ. The method with bathophenanthroline as the iron reagent (*8*) gave similar results, but since there was ample blood available the simpler though less sensitive thioglycolate method was adequate.

Forty-eight hours after being given a single dose of diethylstilbestrol, cockerels incorporate P^{32} into their plasma protein at a rate approaching that in the laying hen (*2*). Correspondingly, the concentration of alkali-labile protein-bound phosphorus is insignificant in plasma of untreated cockerels and high in that of laying hens or cockerels after estrogen treatment (Table 1). Untreated laying hens have a high plasma-iron content and treatment with estrogens causes a tenfold increase in the plasma-iron content of cockerels. The source of this iron, or, in general, the interrelation between the iron content of plasma, liver, and other organs of fowl and the effect of sex hormones is not clear. Although in several vertebrate species including the chicken the female can store more iron in its liver than the male (*9*), treatment of immature pullets with estrogen for 12 days did not increase the concentration of iron in liver (*10*).

Table 1. The phosphoprotein and iron content of the plasma of hens, untreated cockerels, and cockerels treated with estrogen. Forty-eight hours after the subcutaneous inoculation of the indicated substances, the protein was isolated from one sample of plasma and its alkali-labile phosphorus (P) content was estimated (*2*). The iron content of this preparation (shown in parentheses) and of another sample of whole plasma was determined as described in the text. Each value is the mean of duplicate assays on plasma of three animals. DES, diethylstilbestrol; estradiol, estradiol benzoate (Progynon, Schering Corp.).

Substances administered (per 100 g body wt)	Alkali-labile P (µg/ml plasma)	Iron (µg/ml plasma)
	Cockerel	
None	0.5	0.5 (0.7)
	Laying hen	
None	122.5	7.8 (8.0)
	Cockerel	
DES, 5 mg	82.5	5.9 (5.6)
	Cockerel	
Estradiol, 0.3 mg	59.5	5.6 (5.2)
	Cockerel	
Estradiol, 8 µg		2.2

The plasma iron is protein-bound; extraction of the proteins with hot trichloroacetic acid and organic solvents did not remove iron (see numbers in parentheses, Table 1). Prolonged incubation with dilute sodium hydroxide, which splits off the protein-bound phosphate, also removes the iron, whereas dialysis at *p*H 5, which is effective in the case of mammalian transferrin, does not. These observations support the assumption that the accumulated phosphoprotein, by virtue of its iron-binding capacity, is responsible for the increased iron content of plasma in cockerels. Thus, phosvitin appears to become an "iron carrier" while still in the plasma, before it is transferred to the developing egg in the ovary.

To estimate phosphoprotein in plasma, the protein is precipitated and extracted with a series of solvents to remove nonprotein material which might contain phosphorus and is hydrolyzed for 16 hours in dilute alkali. In contrast, estimation of plasma iron is a brief procedure and provides a simple way of evaluating estrogen action. Furthermore, much less estrogen causes a detectable increase in iron than in phosphoprotein.

Figure 1 shows the direct correlation between the amount of injected diethylstilbestrol and the amount of plasma iron detected 18 hours later. Estradiol evokes the same response at concentrations of approximately one-fifth of those of diethylstilbestrol (Table 1). As expected, the injection of urine of pregnant women (1 ml) raises the concentration of iron in the plasma of cockerels by at least 400 percent. As a qualitative test, this method is particularly simple; the difference between the very low iron concentration in the plasma of uninjected cockerels and those injected with material containing more than 4 µg of estradiol can be seen with the naked eye upon the addition of an iron reagent.

The aforementioned approach may conceivably be useful for estimating the estrogen content of biological materials in cases where chemical assay is not applicable. In hens, the concentration of plasma iron may provide a biologically meaningful and easily obtainable measure of sexual maturity and reproductive capacity.

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29 January 1965

Peptide Synthesis from Amino Acids in Aqueous Solution

Abstract. *Four dipeptides and a tripeptide were formed when an aqueous solution of glycine and leucine was exposed to ultraviolet light in the presence of cyanamide.*

In recent years, several experiments have been performed to test the hypothesis of chemical evolution (*1*). The formation of amino acids in such experiments has been widely reported. In his classic experiment on electric discharge, Miller identified the amino acids glycine, alanine, β-alanine, aspartic acid, and glutamic acid (*2*). Palm and Calvin found glycine, alanine, and as-