Full mathematical treatment of these motions has not been given except for the rigid cylinder. However, the main features of the motion can be explained qualitatively in terms of an approximate result given by Schlichting (*2*) and Nyborg (*5*, p. 303). This result, based on a "thin boundary layer" approximation, is expressed in terms of a "limiting" velocity U_L already mentioned, which is characteristic of the streaming velocity near the cylinder just outside the boundary layer. This velocity U_L is parallel to the boundary; we take U_L to be along the x direction, with a choice of meanings for x. Thus for rigid-cylinder streaming (Fig. 2B), x measures arc length along a circle around the wire, perpendicular to the axis. For streaming associated with the flexing vibrations (Fig. 2C), x measures arc length along the wire, parallel to the axis. For tip-associated streaming (Fig. 2D), x measures arc length along a great circle formed by intersecting the hemispherical tip with a plane passing through the wire axis.

Let u_0 be the amplitude of oscillatory irrotational motion (that is, oscillatory motion as it would be in the absence of viscosity) along the x direction. The Schlichting result is then

$$U_L = -(3/8\,\omega)\partial(u_0^2)/\partial x \qquad (3)$$

This expression may be applied qualitatively to any of the situations in Fig. 2.

It can readily be verified that the direction of U_L given by Eq. 3 is in agreement with the direction indicated by arrows near the wire surface in Fig. 2, B and D.

According to Eq. 3 the magnitude of U_L depends on the magnitude of $\partial(u_0^2)/\partial x$ or $2u_0\partial u_0/\partial x$. For purposes of rough comparisons suppose that A is a typical magnitude for u_0 and that in a given situation u_0 decreases from A to zero in a distance l. Then U_L is roughly proportional to A^2/l, and for given A the characteristic streaming velocity U_L varies inversely with l.

For both the situations of (Fig. 2, B and D), the length l may be taken as $\pi a/2$, one-fourth the wire circumference; for the situation of Fig. 2C we choose l as $\lambda/4$. Hence we expect U_L to be of the same order of magnitude for Fig. 2, B and D, while for Fig. 2C the magnitude of U_L will be less by about a factor of $2\pi a/\lambda$. For our typical situation ($a = 0.0125$ cm; $\lambda = 0.9$ cm), this factor is about (1/70). Since the maximum viscous stress near the wire is proportional to U_L, we see that effects of such stresses arise primarily from "cylinder streaming" (Fig. 2B) and tip-associated streaming (Fig. 2D), and not appreciably from the relatively large-scale streaming of Fig. 2C. The last-mentioned is nevertheless significant; it plays the role of transporting suspension from the outer fluid to the high-stress region near the wire; it probably explains the absence of a plateau in Fig. 1 analogous to that noted by Rooney (*1*).

A. R. WILLIAMS
D. E. HUGHES
Department of Microbiology, University College, Cardiff, South Wales
W. L. NYBORG
Physics Department, University of Vermont, Burlington 05401

References and Notes

1. J. A. Rooney, *Science*, this issue.
2. H. Schlichting, *Boundary Layer Theory* (McGraw-Hill, New York, 1955), p. 194.
3. J. Holtzmark, I. Johnson, T. Sikkeland, S. Skavlem, *J. Acoust. Soc. Amer.* **26**, 26 (1954).
4. W. P. Raney, J. C. Corelli, P. J. Westervelt, *ibid.*, p. 1006.
5. W. L. Nyborg, in *Physical Acoustics*, W. P. Mason, Ed. (Academic Press, New York, 1965), vol. 28, chap. 11.
6. P. M. Morse and K. U. Ingard, *Theoretical Acoustics* (McGraw-Hill, New York, 1968), pp. 175 ff.
7. A. R. Williams and W. L. Nyborg, *Ultrasonics* **8**, 36 (1970).
8. The wire was donated by the General Electric Company through R. M. Cogan, Providence, R.I.
9. W.L.N. was Visiting Scientist at the microbiology department, University College, Cardiff, when most of the present work was being done. Supported in part by the Medical Research Council and in part by NIH grant GM-08209.

22 April 1970; revised 26 June 1970

Palladium: Preparation and Catalytic Properties of Particles of Uniform Size

Abstract. *A method has been developed for the preparation of uniform palladium particles of diameter from 55 to 450 angstroms. Uniform particles of gold layered on palladium were also synthesized. Hydrothermal treatment of aluminum hydroxide sol was used to prepare rods of alumina with uniform cross section from 100 to 500 angstroms and of varying lengths. The palladium was adsorbed as individual particles on alumina rods, both present in aqueous suspension. Then the suspension was dried to give a catalyst containing metal particles of uniform size dispersed in open pores produced by the intermeshing of the alumina rods. This procedure guaranteed the absence of diffusion control in the rate of reactions observed experimentally. All stages of the preparation were monitored with the electron microscope. The kinetics of the ethylene-hydrogen reaction were examined by means of a pulse technique. The number of active sites determined by carbon monoxide titration of the surface was equal to the number of surface atoms as determined by the calculation of the quantities of compounds involved in the synthesis and electron microscope examination. Furthermore, the activity per site depended on the method of preparation, being four times smaller when sodium formate was used as a reducing agent instead of sodium citrate. This may be due to the fact that the shape of particles makes certain crystallographic planes more favorable. Decrease in the size of particles to 56 angstroms produced no effect on catalytic activity beyond that due to the increase in the number of surface atoms. The activity of commercial 5 percent palladium on alumina diluted 100-fold with alumina gave 80.4 percent conversion with propylene and 82.7 percent conversion with ethylene. Thus there was little difference in the behavior of the two olefins.*

The relation between particle size and catalytic activity is of basic importance in heterogeneous catalysis. It is of great theoretical importance to determine whether the catalytic activity increases continuously as one increases the surface, whether there is a preferred size for maximum activity, or whether the catalytic activity disappears at a size at which the metallic properties of the particles also disappear. Furthermore, comparison of variation of chemical composition, catalyst support, and nuclear irradiation can be carried out more systematically if the particle size is kept constant. It is also of practical importance to be able to synthesize catalysts of maximum catalytic activity and to understand and to control each step of the catalyst preparation so as to ensure reproducible catalytic activity.

It was felt that the knowledge of systems and synthesis of colloidal gold (*1*) could be used as a guide in the preparation of palladium catalysts of desired uniform particle size and shape. In addition, the microtesting technique (*2*) would afford one an elegant means of characterizing the catalysts so prepared. We report here the hydrogenation of ethylene on palladium catalysts of varying sizes supported on specially synthesized alumina plates.

Several authors have conducted preliminary examinations of the relationship between catalytic activity and particle or crystallite size. Rampino *et al.* (*3*) studied the hydrogenation of nitrobenzene with palladium particles supported on polyvinyl alcohol. The particle size was determined with an ultracentrifuge. Although some micrographs were included, these were not used to determine the particle size distribution of the palladium. The smallest particle size used was 90 Å in diameter. It was found that the catalytic activity increased with a decrease in particle size. Ivannikov *et al.* (*4*) and Natta and Agliardi (*5*) found that an increase in the crystallite size of a zinc oxide catalyst causes a decrease in the catalytic activity. In recent years a number of studies have been carried out on the specific activity of catalysts, notably those of Boudart and his collaborators (*6*).

The requirements placed on catalyst preparation were that it produce in solution particles of uniform size and shape, that these be adsorbed on a support in a uniform way without clumping, and that this support be of such a nature that the catalyst particles be readily available to the reactants. It was therefore essential to determine those factors which determine the size, size distribution, and stability of colloidal palladium and alumina and the interaction between these two systems.

Table 1. Effect of preparation conditions on the size of the palladium particle.

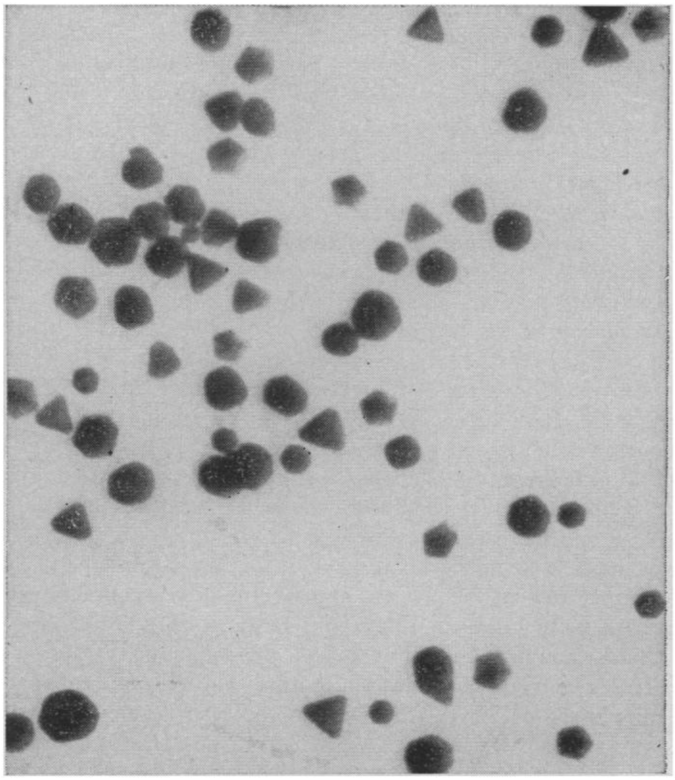
Diameter of particle (Å)	Sodium formate (ml)	NaOH (ml)	*p*H	Time (hours)
470	9.5	0		180
450	9.5	0		32
325	9.5	25	2.1	52
275	12.0	0	3.1	38
275	12.0	0		38
250	9.5	5		32
250	20	30	4.3	17
250	100	60		½
250	120	30	7	1
200	100	45	11.2	1
200	100	45	11.1	1
125	12	30		3

Investigators have prepared hydrosols of the palladium family of metals in a variety of ways. These include the Bredig arc method (*7*) and the Kunkel peptization method (*8*). The following materials have been used as reducing agents: hydrogen (*3*), formaldehyde (*9*), formaldehyde and sodium citrate (*10*), carbon monoxide (*11*), hydrazine hydrate (*12*), sodium formate (*13*), acrolein (*14*), titanium chloride (*15*), and hydrazine hydrate with sodium protalbinate, sodium lysalbinate, or Iceland moss as protective colloid (*16*).

The above preparations of colloidal palladium described in the literature were not characterized by electron microscopy with respect to particle shape, size, or aggregation and were synthesized by surface decomposition. Furthermore, since the stability of the hydrosols of the metals of the platinum family is much lower than that of colloidal gold, particular care must be taken not to ascribe a negative or an irreproducible result to an unsatisfactory synthesis, when in fact the trouble may be the result of rapid coagulation of an otherwise successful particle preparation.

In the present investigation the reducing agents used were sodium formate, sodium citrate, sodium acetone dicarboxylate, and hydrogen. Sodium polyacrylate (SPA) and sodium citrate were used as stabilizing agents. Both spectrophotometric and electron microscopic examinations were made of the preparations.

The particle shape, size, size distribution, and state of aggregation were determined by means of an electron microscope (RCA-MU-2A). The method of preparation of the sample for examination depended on the nature of the palladium sol. For sols stabilized with sodium polyacrylate, a drop of



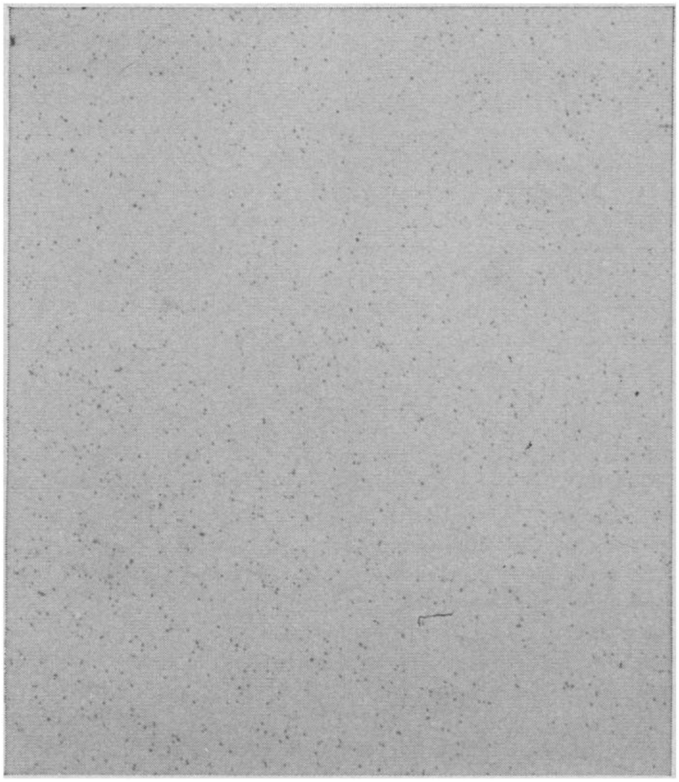


Fig. 1 (left). Colloidal palladium particles (average size, 450 Å) made by reduction with sodium formate. Fig. 2 (right). Colloidal palladium particles (average size, 75 Å) made by reduction with sodium citrate.

the sol was placed on the collodion membrane and allowed to evaporate. Particles of palladium could be seen attached to the fibers of the sodium polyacrylate. The stability of the sodium formate–sodium polyacrylate–palladium sol is undoubtedly due to this adsorption. This method of preparation and mounting is unsatisfactory for palladium particles smaller than 100 Å since the latter are difficult to see against a background of the sodium polyacrylate fiber. For other sols the solution was protected from clumping by the addition of a 0.1 percent gelatin solution to make the colloid 0.01 percent in gelatin. The colloid (3 ml) was centrifuged for 20 minutes in an air-driven centrifuge at 10,000 rev/min in order to drive the particles onto a collodion screen. The screen was washed with water and the last droplet was removed by a small capillary. Very small particles, which are difficult to see in the electron microscope, were grown in a controlled way to a size that could be readily examined in the microscope. From the particle size distribution so obtained the particle size distribution of the original sol could be calculated. A spectrophotometer (Beckman D-U) was used to study the changes in both visible and ultraviolet absorbance during the course of synthesis of the palladium sols.

Palladium chloride stock solution was made by dissolving anhydrous palladium chloride in 3*N* HCl. Palladium–sodium formate–sodium polyacrylate sol was prepared in the following way: 840 ml of water, 60 ml of 0.2 percent SPA (Rohm and Haas, GS grade), and 12 ml of 0.28 percent palladium chloride solution were treated with sodium hydroxide to obtain the desired *p*H value. The solution was heated to gentle reflux. After the addition of an appropriate amount of 1 percent sodium formate solution with vigorous manual agitation, the solution was refluxed until the reduction was completed. By changing the conditions of the reduction, particularly the amount of the reducing agent and the *p*H of the solutions, it was possible to obtain uniform particles of palladium in the size range from 125 to 470 Å (Table 1). A typical preparation is shown in Fig. 1 (*17*). As the solution becomes more alkaline, the diameter of the particle decreases.

Preliminary experiments on the preparation of a palladium–hydrogen–sodium citrate sol gave erratic results: Either no reduction took place or reduction took place with the coagulation of the sol. The fact that the citrate ion acts as a stabilizer was shown in a set of experiments in which hydrogen was bubbled at 50°C through a $1.86 \times 10^{-4}M$ $PdCl_2$ solution to which various concentrations of sodium citrate had been added. A ratio of 75 citrate ions per palladium ion gives satisfactory stability to the palladium sols. That hydrogen gas and not sodium citrate is the reducing agent at 50°C is shown by the fact that a solution $1.86 \times 10^{-4}M$ in palladium chloride and 7 m*M* in citrate does not show any color change after 4 hours at 50°C.

Table 2. Catalytic activity as a function of particle size.

Sample No.	Diameter (Å)	Pd content (ppm)	Percentage conversion per 25 mg catalyst	Relative activity	
				Per unit wt. Pd*	Per unit surface
64	55	170	50	294	12.8
64	55	170	45	265	11.5
59	75	250	45	180	10.6
60	75	200	43	215	12.7
60	75	200	40.5	203	11.9
63	115	250	38.5	154	14.0
73	145	250	29	116	13.5
24	125	940	33	35.1	3.5
28	250	830	21	25.3	5.0
29	450	770	6	7.8	2.8
			Baker catalyst		
	(70)	(5%)		147	7.3

* The relative activity per unit weight refers to 25 mg of alumina catalyst with 0.1 percent palladium by weight.

Particle size increases with time of reduction. In the later stages of the reduction growth took place by aggregation of particles rather than by continuous deposition of palladium particles already formed. Furthermore, prolonged passage of hydrogen caused precipitation of the colloidal palladium. Thus special care must be taken to keep the palladium from precipitation when a suspension of colloidal palladium is used for hydrogenation reactions.

Palladium–sodium citrate sols were found to be the most stable hydrosol of palladium. The standard sol, which gives particles 75 Å in diameter, was prepared in the following way: 100 ml of $9.3 \times 10^{-4}M$ $PdCl_2$ solution (prepared by dissolving 0.165 g of anhydrous $PdCl_2$ in 20 ml of 1*N* HCl and adding enough water to make 1 liter of solution) was mixed with 200 ml of $3.4 \times 10^{-2}M$ (1 percent by weight) sodium citrate solution in a liter flask. The solution was diluted to 500 ml and was refluxed in an electric heating mantle for 6 hours. The olive brown color characteristic of the sol was noticeable only after 4 hours of refluxing. If the concentrations of the reactant solutions are increased, the size of the palladium particles increases (Fig. 2).

The particle size distribution of the palladium sols obtained in this way is given in Fig. 3. It is seen that, whereas the standard concentration produced

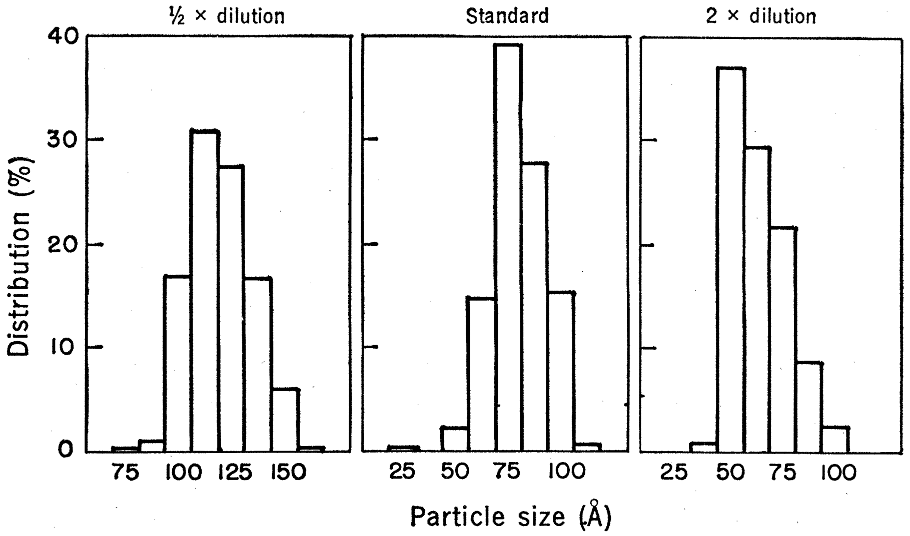


Fig. 3. Size distribution of palladium particles as a function of preparative conditions.

Table 3. Surface area of the palladium catalyst.

Sample No.	Electron microscope values (m^2/g)	Chemisorption values (m^2/g)
60	60	67
73	33	32
25	15	11
29	11	8.8
Baker 5% Pd		73
Baker 5% Pd		84

particles 75 Å in diameter with a symmetrical shape, the more diluted solution with smaller particles had a distribution containing more of the large particles, an indication that the larger particles grow faster than the smaller ones. This is consistent with the observations made on colloidal gold.

The *p*H of the reacting solution was also varied in the otherwise standard palladium–sodium citrate system. In the 5.1 to 5.8 *p*H range, single 150-Å particles and aggregates of these were produced. At a *p*H of 6.1, the standard condition, uniform particles of 75 Å were observed with no aggregates. In the 6.4 to 6.9 *p*H range, the majority of particles were 500 Å in size with some as large as 1200 Å and with some 75 Å in diameter still present. The larger particles looked like pseudocrystals or regular aggregates of smaller particles. At a *p*H of 8, the appearance of the palladium particles is very similar to that produced in the 6.4 to 6.9 range with almost no fine particles and with the larger particles having the appearance of true crystals with sharp edges. The lower the *p*H in the range of 5 to 8, the faster the color change.

Undialyzed palladium sols obtained by the use of sodium formate, sodium polyacrylate, or sodium citrate as reducing agents gave a spectrum with a maximum in absorption at 260 nm. The colloidal solution is brown. Spectra of the undialyzed sol show that the position of this absorption maximum does not change with particle size. The sodium polyacrylate–palladium sol dialyzed to a resistance of 12.5 kilohms retained its spectrum, whereas the sodium citrate–palladium sol on dialysis to 4.6 kilohms showed a decrease in absorption undoubtedly due to coagulation.

Palladium chloride–sodium acetone dicarboxylate sol was prepared as follows: 100 ml of acidified $9.31 \times 10^{-4}M$ palladium chloride solution and 200 ml of $3.40 \times 10^{-2}M$ sodium acetone dicarboxylate solutions were diluted to 500 ml and refluxed in the same way as in the preparation of the standard sodium citrate sol of palladium. The color change was complete in 35 minutes, a much shorter time than that required for reduction by sodium citrate alone. Uniform particles 75 Å in diameter were produced. Since acetone dicarboxylic acid is more effective in the reduction and is a known oxidation product of sodium citrate oxidation, it may be the actual reducing agent in the standard sodium citrate reduction of palladium. The palladium sol obtained in the preparation was unstable and underwent complete precipitation in about a month.

Table 4. Poisoning of catalytic activity of palladium by carbon monoxide.

Molecules of CO introduced ($\times 10^{13}$)	Surface covered (%)	Surface empty (%)	Relative catalytic activity
0	0	100	100
2.4	2.0	98	98
6.2	5.3	94.7	96
8.2	7	92.7	92
11.2	9.4	90.6	89
14.6	12.6	83.4	85

However, with a mixture of 100 ml of $3.40 \times 10^{-2}M$ sodium citrate solution and 100 ml of $3.40 \times 10^{-2}M$ sodium acetone dicarboxylate solution it was readily possible to make a sol that is as stable as the standard sodium citrate with an average diameter of particles of 75 Å.

A method was developed for growing

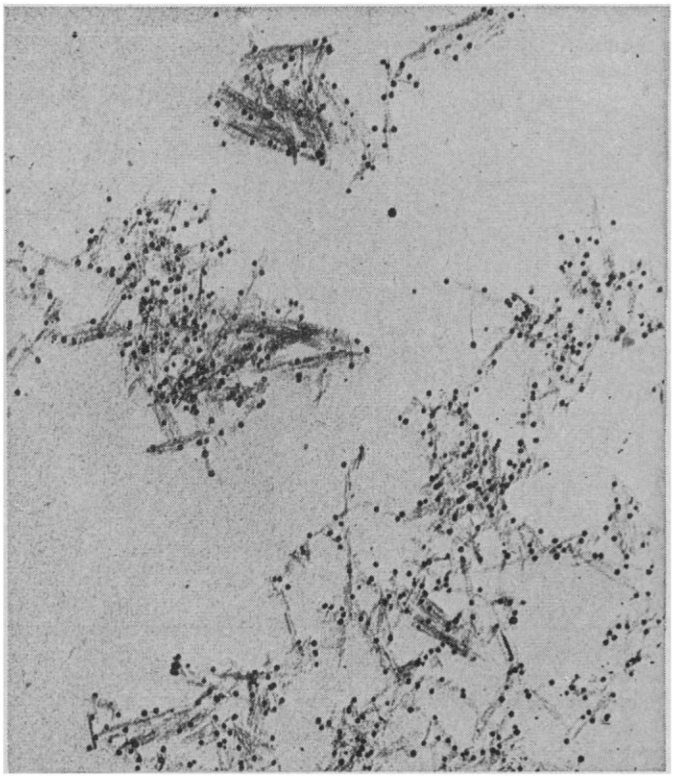


Fig. 4 (left). Palladium catalyst, 75-Å palladium particles, on boehmite alumina rods.

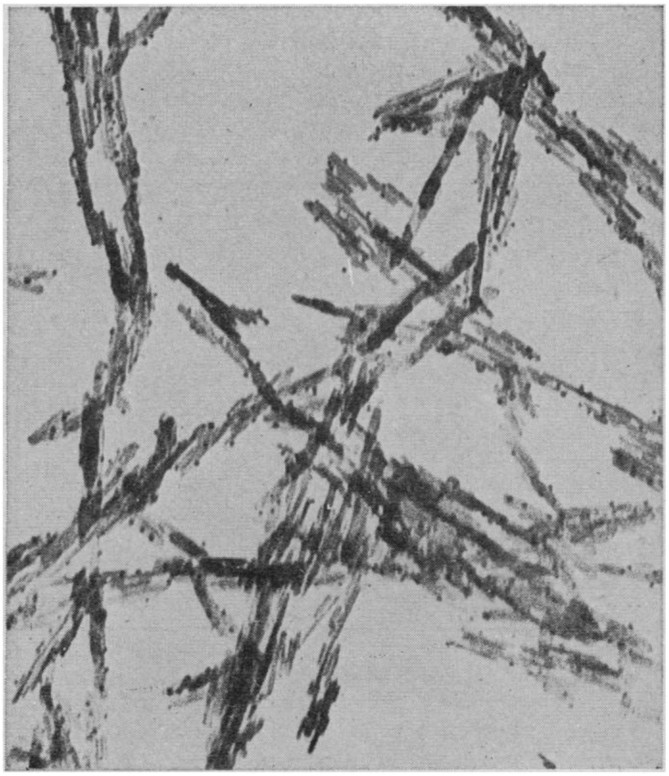


Fig. 5 (right). Palladium catalyst, 75-Å palladium particles, on Baymol plates.

in a controlled way palladium on palladium in order to produce particles of desired size and to study the growth process. Because of its use in the growth of gold particles, hydroxylamine hydrochloride seemed the proper reducing agent for the growth of palladium particles. If the *p*H is maintained between 3.3 and 6.1, the palladium deposits on the palladium particles at room temperature. Below a *p*H of 3, no appreciable reduction took place whereas, above a *p*H of 6.3, spontaneous nucleation was evident. The details of the procedure are as follows. A standard sodium citrate–palladium sol of particles 75 Å in diameter (25 ml) at a *p*H of 6.1 was diluted to 100 ml; 70 ml of $4.65 \times 10^{-4}M$ palladium chloride solution and 70 ml of $6.25 \times 10^{-3}M$ hydroxylamine hydrochloride solution were added at room temperature from two separate dropping funnels at the rate of 4 ml/min. The solution was agitated with a magnetic stirrer. During the process of addition the *p*H decreased from 6.1 to 3.3. Examination of the resulting solutions in the electron microscope showed that most of the particles were aggregated. The sol was less stable than that prepared by the standard citrate method, and the stability of the sol diminished as the initial *p*H of the nucleating sol decreased. Stability was attained by adding sodium citrate until the concentration of the sol was $2 \times 10^{-3}M$ or twice that of the standard sol. The average diameter of the particles in the grown sol D_f can be calculated from the formula

$$D_f = D_0\left(1 + \frac{M_1}{M_0}\right)^{1/3}$$

where D_0 is the average diameter of the original palladium nucleus, M_0 is the mass of the palladium present in these nuclei, and M_1 is the mass of the palladium added as an ion. The average diameter of the palladium particles used as nuclei was 75 Å, that of those doubled in size with palladium was 150 Å, and that of those quadrupled was 300 Å. Thus satisfactory control over growth was obtained.

The procedure that was used to grow palladium on palladium was modified for the growth of gold on palladium. In this case the palladium chloride used in that procedure was replaced by an equivalent amount of chlorauric acid. If one starts with palladium particles 60, 75, or 115 Å in diameter, doubling the size with gold leads to particles 125, 150, and 250 Å in diameter, respectively, and a second doubling of the size with gold produces particles 240, 300, and 460 Å in diameter, respectively. Although the resulting sols were found to have many aggregates, the stability of the gold-layered palladium sols was much greater than that of those produced by growing palladium on palladium. Controlled deposition of gold on palladium was used successfully to determine the average size and particle size distribution of extremely fine-particle palladium sols.

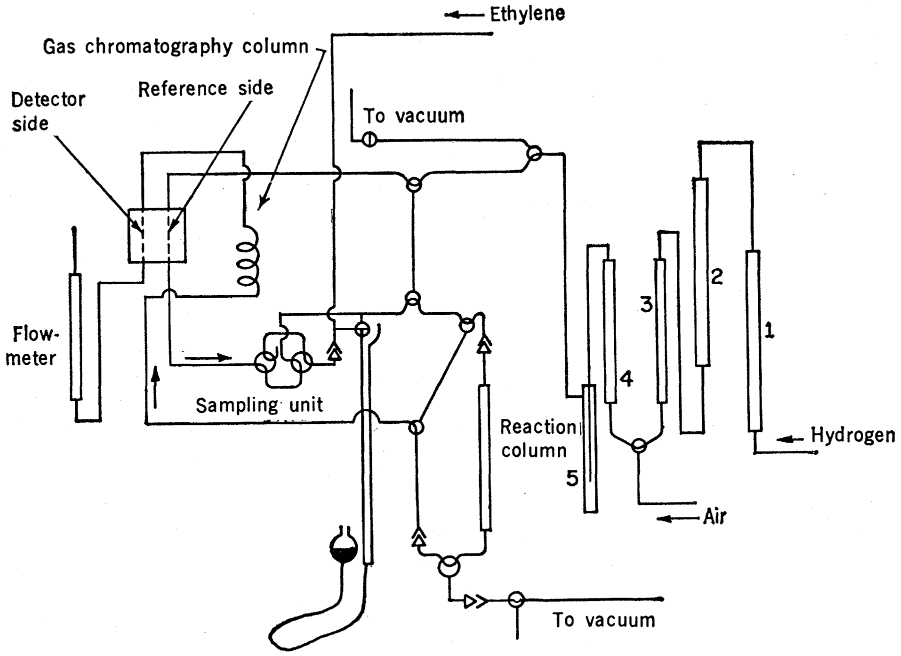


Fig. 6. Apparatus for pulse catalytic studies.

Gamma alumina was prepared in the following way. Distilled water (700 ml) was refluxed in a 2-liter three-necked flask. A solution containing 13.5 g of aluminum isopropoxide in 250 ml of isopropyl alcohol was added into this boiling solution at a rate of 55 ml/min. The mixture was refluxed for 1½ hours with constant stirring and then for an additional 2 hours after the heating mantle was turned off. The resulting white turbid suspension was washed three times by decantation. The alumina sol obtained was converted into gamma alumina by drying at 110°C for 24 hours and at 350°C at a pressure of 10^{-4} mm-Hg for 4 hours.

Two methods were developed for the preparation of the alumina support for the palladium catalysts. In one preparation 80 ml of a 1 percent suspension of fibrillar Baymol (E. I. du Pont de Nemours & Co., technical grade) were autoclaved for 2 hours at 220°C to obtain rods 110 Å wide and 1000 Å long. Before being used as a support for the catalyst, the suspension was treated with a mixed bed resin (Rohm and Haas MB-1) until it showed a resistance of 35 kilohms (*18*). Another preparation of platelike boehmite was obtained by autoclaving 65 ml of a suspension of alumina sol with 5 ml of 1*N* acetic acid for 2 hours at 220°C. The products were plates 200 Å wide and several thousand angstroms long.

Palladium sols were added to the boehmite rod suspensions with constant stirring. Electron micrographs show that the negatively charged palladium particles are completely adsorbed on the positively charged surfaces of the boehmite rods. There were no palladium particles in the background (Fig. 4). The suspension so obtained was dried in a flash evaporator and then at 110°C for 48 hours. The catalyst was pulverized to between 60 and 80 mesh and dried at 350°C for 4 hours at a pressure of 10^{-4} mm-Hg. Particles of 80 to 100 mesh were used for catalytic studies and were analyzed for palladium (*19*); *p*-nitroso diphenylamine was used as an indicator, and the absorption was determined on a spectrophotometer (Beckman model B). A similar procedure was used to deposit palladium particles on Baymol rods (Fig. 5).

Catalytic runs were carried out in the following way: Ethylene was purified by distillation from a liquid ethylene trap and was stored in a 3-liter reservoir flask. A manometer was used as an indicator of the pressure of the purified ethylene. A known amount of purified ethylene was trapped in a 3.97-

ml gas trap consisting of two four-way stopcocks. The stopcocks could be rotated to permit the carrier hydrogen gas to drive the trapped ethylene into the reaction column. The pressure of the trapped ethylene was measured on a manometer (Fig. 6).

The carrier hydrogen was purified by passage through a deoxygenation unit (Deoxy Hydrogen Purification Unit, Engelhardt Industries, East Newark, N.J.), silica gel, Drierite, and a trap cooled to liquid-nitrogen temperatures inserted into the flow system. The flow rate was controlled by a two-stage pressure regulator, a reservoir, capillary tubing, a soap-bubble flowmeter, and a wet-test meter. The reaction column was a Pyrex glass tube 8 mm in outside diameter with ground glass joints at both ends.

An iron-constantan thermocouple was inserted into a Pyrex glass well 4.5 mm in outside diameter so that its tip was just in the middle of the catalyst layer. The reactor could be heated by a small electric furnace which could be moved up and down along the reactor. The temperature of the catalyst bed was controlled by either a water jacket or a furnace around the reactor, and the temperature was determined both by a thermocouple pyrometer (Engelhardt) and by a thermometer placed just outside the catalyst bed. A three-way stopcock placed at the exit of the reactor permitted gas flow either to the vacuum system or to the gas chromatographic assembly.

The vacuum system consisted of a mercury-diffusion pump and a mechanical backing pump. The vacuum was measured either on a manometer or on a thermocouple-type vacuum gauge (RCA 1946).

The gas chromatographic system (Aerograph, Wilkens Instrument and Research Inc.) consisted of a thermal conductivity cell with tungsten filaments, a chromatographic column (152.5 by 0.64 cm) of activated silica and a recorder (Brown) with 12-mv range.

All the catalysts prepared were found to be so active that dilution by the addition of 30 times as much gamma alumina was necessary in order to check their activity for hydrogenation of ethylene at 0°C. An 0.850-g portion of diluted catalyst sample was placed in a reactor, thus forming a bed about 20 mm thick.

Activation was carried out before each catalytic run to eliminate any organic material, oxygen, or moisture that might have contaminated the catalyst. For this purpose the catalyst was gradually warmed up to 350°C in a vacuum for 2 hours, treated with dry air for 40 minutes at a rate of 60 ml/min, evacuated for 40 minutes, treated with hydrogen for 40 minutes at 60 ml/min, evacuated for 1 hour, and treated again with hydrogen for 1 hour. The hydrogen stream was discontinued while the catalyst temperature was lowered to room temperature.

The catalyst was brought to a temperature of 0°C by means of an ice bath, and a stream of hydrogen was allowed to flow through the thermal conductivity cell at 38 ml/min. A sample of 6.26×10^{19} molecules of ethylene was introduced into the hydrogen stream from the sample trap containing 3.97 ml of gas at a pressure of 48 mm-Hg.

The separation of the unconverted ethylene from ethane by a silica column at 35°C was satisfactory. The retention times of ethylene and ethane were 7 and 5 minutes, respectively. Calibration was made for both ethane and ethylene, and the electrical conditions were adjusted so that the peak area of ethane (A_1) was the same as that of ethylene (A_2).

The percentage conversion was calculated by the formula $100\ A_1/(A_1 + A_2)$. This percentage conversion was taken as a measure of the catalytic activity of the catalyst.

Experimental results obtained in the hydrogenation of ethylene at 0°C are given in Table 2. The activity per unit surface of particles obtained by the sodium citrate method (samples 59, 60, 63, 64, 73) is higher by a factor of 4 than those of particles prepared by the sodium formate method. However, in both cases the activity is proportional to the surface. Thus there are no special active centers on the surface; every atom on the surface is active and has equal activity. The difference between the two groups is one that affects all the atoms on the surface. It may be due to the different shape of the smaller particles of the sodium citrate preparation, which results in the development of different crystallographic faces. The Baker commercial catalyst had an intermediate value for activity per surface site.

Furthermore, no dramatic change in the activity was observed as one approached the limit of the smallest particle used, 55 Å. If the cause of catalytic activity is a pool of metallic electrons, there is a sufficient number of such electrons in this particle of 4000 palladium atoms to constitute such a pool. This particle must still be a metal.

A comparison was made of the hydrogenation of ethylene and propylene in which 29.1 mg of 5 percent palladium on alumina (Baker) diluted 100 times with alumina was used. There was little difference in the behavior of the two olefins; 6×10^{19} molecules of ethylene gave 82.7 percent conversion whereas the same number of propylene molecules gave 80.4 percent conversion.

Chemisorption of carbon monoxide was carried out to determine whether the area of the surface after activation and use in a chemical reaction was the same as that calculated from electron microscopic determinations of the palladium particles prior to their being mounted on the catalyst support.

The determination of the degree of chemisorption was carried out in the following way in the apparatus used for catalytic studies. The limit of detection of carbon monoxide in the hydrogen stream was lowered to 6.4×10^{15} molecules by amplifying the output of the thermal conductivity cell with a d-c amplifier (Kintel model 121A). A number of aliquots containing this number of carbon monoxide molecules were introduced into the hydrogen stream in the same way as the ethylene was introduced. The results shown in Table 3 were calculated on the basis of one carbon monoxide molecule adsorbed on one surface palladium atom. The reproducibility of the procedure was determined by measuring the chemisorption of carbon monoxide on 5 percent palladium on alumina (Baker catalyst) on the different samples diluted with gamma alumina. This is seen to be about 13 percent. Table 3 shows that satisfactory agreement for the surface is obtained by these two methods. Furthermore, the agreement between the two results shows that the chemisorption of carbon monoxide is linear rather than of the bridge type (*20*).

The poisoning experiments were carried out by injecting into a hydrogen stream passing over the catalyst [in sample 64 (Table 2) palladium particles were 55 Å in diameter] alternate pulses of ethylene and carbon monoxide. The first pulse of ethylene gave 65 percent conversion to ethane. With each successive addition of carbon monoxide, the percentage conversion of ethylene to ethane was reduced while the carbon monoxide was retained on the surface. The results (Table 4) represent a partial titration of the active surface. It is seen that the catalytic activity for ethylene hydrogenation is proportional to the percentage of surface free from carbon monoxide. Thus there is no evidence of special active sites in the smallest particle (55 Å) examined.

JOHN TURKEVICH
GWAN KIM

Department of Chemistry,
Princeton University,
Princeton, New Jersey

References and Notes

1. J. Turkevich, *Amer. Sci.* **47**, 97 (1959); ———, J. Hillier, P. C. Stevenson, *Discuss. Faraday Soc.* No. 11, 55 (1951).
2. R. J. Kokes, H. Tobin, Jr., P. H. Emmett, *J. Amer. Chem. Soc.* **77**, 5860 (1955).
3. L. D. Rampino, K. E. Kavanagh, F. F. Nord, *Proc. Nat. Acad. Sci. U.S.* **29**, 246 (1943).
4. P. Ya. Ivannikov, A. V. Frost, M. I. Shapiro, *C. R. Acad. Sci. USSR* **1933**, 124 (1933).
5. G. Natta and N. Agliardi, *Atti Accad. Naz. Lincei Mem. Classe Sci. Fis. Mat. Nat. Sez. VIII* **11**, 383 (1947); *ibid.*, p. 500.
6. M. Boudart, A. Aldag, J. E. Benson, N. A. Dougharty, C. G. Harkins, *J. Catalysis* **6**, 92 (1966); G. K. Boreskov and A. P. Karnauktev, *Zh. Fiz. Chim.* **26**, 1814 (1952); G. K. Boreskov and V. S. Chesalova, *ibid.* **30**, 2560 (1956); G. K. Boreskov, M. G. Slin'ko, V. S. Chesalova, *ibid.*, p. 2757; M. A. Abdenko, G. K. Boreskov, M. G. Slin'ko, *Probl. Kinetiki Kataliza Akad. Nauk SSSR* **9**, 61 (1957).
7. G. Bredig and N. Fortner, *Chem. Ber.* **37**, 798 (1904); S. Miyamoto, *J. Chem. Soc. Jap.* **55**, 611 (1934); ———, *Kolloid Z.* **67**, 284 (1934).
8. A. F. Benton, *J. Phys. Chem.* **30**, 1415 (1926).
9. A. Lottermoser, *Amer. J. Sci.* **16**, No. 4, 381 (1903).
10. K. Shigena, *Osaka Furitsu Kogyo-Shoreikan Hokoku* **8**, No. 2, 1 (1927).
11. J. Donau, *Monatsh. Chem.* **26**, 525 (1905).
12. A. Gutbier, *Z. Anorg. Chem.* **32**, 347 (1902).
13. H. Wieland, *Chem. Ber.* **45**, 484 (1912).
14. N. Castoro, *Z. Anorg. Chem.* **41**, 126 (1904).
15. A. Gutbier and H. Weithase, *Z. Anorg. Allg. Chem.* **169**, 264 (1928).
16. C. Paal and C. Amberger, *Chem. Ber.* **37**, 124 (1904); *ibid.* **40**, 1392 (1907); A. Gutbier, J. Huber, E. Kuhn, *Kolloid Z.* **18**, 57 (1916); V. P. Tsybasov, G. A. Dymshits, K. M. Dvorkina, *Khim. Tverd. Topl.* **7**, 71 (1936); N. A. Gruzdeva, *Zavod. Lab.* **15**, 1247 (1949).
17. Preliminary work on the preparation was carried out by Dr. P. Fox in this laboratory.
18. J. Bugosh, U.S. Patent 2,915,475 (1 Dec. 1959).
19. E. B. Sandell, *Colorimetric Determination of Traces of Metals* (Interscience, New York, ed. 3, 1959), p. 714; J. H. Yoe and L. G. Overholser, *J. Amer. Chem. Soc.* **61**, 2058 (1939).
20. R. P. Eischens, S. A. Francis, W. A. Pliskin, *J. Phys. Chem.* **60**, 194 (1956).

25 February 1970; revised 13 July 1970 ■

Thyroid Hormone Control of Erythrocyte 2,3-Diphosphoglyceric Acid Concentrations

Abstract. *A biphasic thyroid hormonal effect has been shown on 2,3-diphosphoglyceric acid synthesis in a crude enzyme, hemoglobin-free preparation from normal human erythrocytes.*

An increase in dissociation of oxygen from hemoglobin occurs on the addition of 2,3-diphosphoglyceric acid (2,3-DPG) to hemoglobin in free solution (*1*). A decrease in the oxygen affinity of hemoglobin in blood of hyperthyroid patients as well as in euthyroid man and rats occurs after treatment with 3,5,3-triiodo-L-thyronine (T3) (*2*). These findings could explain the shift to the right in the oxygen dissociation curve with a subsequent increase in oxygenation of the peripheral tissues. Recently, 2,3-DPG has been shown to be increased in patients with hyperthyroidism (*3*) as well as in normal red cells incubated with T3 (*4*).

To investigate whether the thyroid hormonal effect on intact cells was on the red cell membrane, on a specific enzyme, or on a group of enzymes, a crude enzyme preparation from human erythrocytes was prepared. Seven-day-old whole blood (200 ml), collected in ACD (citric acid, trisodium citrate, and dextrose), was washed three times in ice-cold saline; the packed cells were adjusted to a hematocrit of 35 to 40 percent with a Kreb's-Ringer bicarbonate buffer (*p*H 7.4) containing $10^{-4}M$ mercaptoethanol and $10^{-5}M$ ethylenediaminetetraacetate. These cells were frozen and thawed three times, and the stroma were removed by centrifugation at 30,000*g* for 30 minutes. The stroma-free hemolysate was then mixed with an equal volume of a suspension of 10 percent diethylaminoethyl (DEAE) cellulose in 3 m*M* potassium phosphate buffer (*p*H 7.26); the mixture was allowed to sit at 4°C for 30 minutes and was then centrifuged. The hemolysate was discarded and the DEAE-cellulose suspension was repeatedly washed with an equal volume 3 m*M* potassium phosphate buffer (*p*H 7.26) until the supernatant was colorless. The enzymes were eluted from the resin with 0.5*M* KCl (two 50-ml volumes). The supernatant, free of hemoglobin and rich in enzyme, was then dialyzed overnight against 0.15*M* KCl (*p*H 7.26) containing $10^{-4}M$ mercaptoethanol. The following day 2.5 mg of bovine serum albumin per milliliter of partially purified enzyme fraction was added, and diphosphoglyceromutase was assayed (*5*). The enzymatic assay for 2,3-DPG was performed by Beutler's modification (*6*) of Krimsky's method (*7*); 1,3-diphosphoglyceric acid and 3-phosphoglyceric acid were assayed by the methods of Negelein (*8*) and were either absent or not detectable. In the crude enzyme preparation, the 2,3-DPG concentrations were 5 to 7 nmole/ml.

The crude enzyme preparation containing diphosphoglyceromutase was incubated in a system which generated 1,3-diphosphoglyceric acid. The system contained (in a total volume of 2.2 ml) glyceraldehyde-3-phosphate (20 μmole), nicotinamide-adenine dinucleotide (10 μmole), 3-phosphoglyceric acid (0.1 μmole), $MnCl_2$ (1 μmole), potassium phosphate (*p*H 7.5) (2 μmole), lactic dehydrogenase [4 international units (I. U.)], potassium pyruvate (40 μmole), glyceraldehyde-3-phosphate dehydrogenase (5 I.U.), triethanolamine-hydrochloride buffer (*p*H 7.8) (100 μmole), and mercaptoethanol ($10^{-4}M$), with a final *p*H of 7.6 to 7.7. Varying concentrations of L-thyroxine (T4) and T3 were added, and the preparation was incubated for 1 hour (Fig. 1). Stock solutions of the thyroid hormones were prepared by diluting T4 or T3 in water distilled twice in glass just before use to a concentration of $3.75 \times 10^{-4}M$. A few drops of 1*N* NaOH were added, and the solution was heated to 40°C to increase solubility. Subsequent dilutions were made from this stock. The results show a biphasic effect on 2,3-DPG synthesis. The stimulatory effect was first witnessed with T3 at concentrations of $3.75 \times 10^{-14}M$ and was maximum at $3.75 \times 10^{-8}M$; the effect decreased with increasing concentrations of thyroid hormone. The effect of T4 was noted initially at a concentration of $3.75 \times 10^{-12}M$ and peaked at $3.75 \times 10^{-10}M$.

Astrup *et al.* (*9*) recently pointed out that no biochemical explanation has been presented for the shifts in oxygen dissociation curve observed in thyroid disorders by Gahlenbeck and Bartels (*2*).

We have demonstrated a direct effect on the concentration of 2,3-DPG in the red cells by thyroid hormone. In view of previous findings (*1*) in conjunction with those discussed above, a biochem-