REPORTS

On the Mechanism of Diamond Formation

Diamonds form by the shock-conversion of graphite, but not by decomposition of metal carbides.

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In an earlier report (*1*) we concluded, on the basis of a metallographic study, that the diamonds in the Canyon Diablo meteorite were formed during impact with the earth. Specifically, we suggested the following alternative mechanisms for diamond formation:

1) The direct, solid-state transformation of graphite by the impact shock.

2) The growth of diamond from an iron carbide, iron sulfide melt (i) as a stable phase under the high pressures of the impact shock; (ii) as a stable phase under localized stresses (these could result either from the unequal thermal expansion of cohenite [meteoritic Fe_3C], kamacite [α-iron], or troilite [FeS] or from the volume expansion on fusion of troilite); or (iii) as a metastable phase under localized stresses like those listed above except that the maximum pressure would be below the graphite-diamond transition pressure.

In regard to the laboratory production of diamond, we also pointed out that formation of diamonds should be thermodynamically possible not only through the decomposition of iron carbide melts but also through the solid-state decomposition of cementite (Fe_3C) and thermodynamically similar metal carbides under high static pressures.

Recently, the direct, solid-state transformation of graphite was verified by DeCarli and Jamieson (*2*), who succeeded in converting graphite to diamond by shock.

The first two mechanisms proposed for the growth of diamond from an iron carbide, iron sulfide melt are essentially similar to the General Electric Co. process of diamond synthesis (*3*) and, except for identification of the sources of pressure, may therefore be regarded as confirmed. In this report we describe results of experiments designed to test (i) the third mechanism proposed for the growth of diamond from an iron carbide, iron sulfide melt and (ii) the solid-state decomposition of metal carbide under high static pressures.

In the first series of experiments we attempted to grow diamonds metastably by allowing iron carbide, iron sulfide melts to decompose spontaneously at atmospheric pressure. In the second series, we subjected solid iron carbides and nickel carbides to static pressures above the diamond-graphite equilibrium line for extended periods. The rationalization underlying both groups of experiments is illustrated in Fig. 1 [Fig. 2 of our previous report (*1*), which gives the stability fields of graphite, diamond, and cementite in the presence of excess α-iron, with the pressure and temperature conditions of our experiments indicated].

As pointed out previously (*1*), the abscissa of this figure may be in error by as much as 300°K because of an uncertainty in the standard enthalpy of formation of cementite. Indeed, commonly accepted values of temperature *B* (the temperature, at a pressure of 1 atmosphere, at which the free energy of decomposition of cementite to γ-iron and graphite equals zero) lie from 200° to 400° to the right of the point shown in Fig. 1 (*4*). For this reason our experiments on cementite were conducted in the region to the right of the calculated cementite stability line.

We further pointed out in our earlier report that this figure is illustrative of the general phase diagram describing the decomposition of all metal carbides having small, positive free energies of formation. Because of its low thermodynamic stability, Ni_3C [$\Delta H^\circ_f = 11$ kcal/mole, as compared with 5.0 kcal/mole (*5*) for Fe_3C] looked particularly promising for investigation and was therefore included in the study reported here.

The failure of diamond to form under conditions where it is thermodynamically stable can be due to slow nucleation, slow crystal growth, or some combination of these factors. The crystal-growth process is usually diffusion-controlled, and thus semiquantitative estimates of expected growth rates can be made. Nucleation rates are more difficult to predict, however, and we therefore attempted to eliminate this factor in some of our experiments by seeding with diamond. To establish whether any growth had taken place, we included some C^{14}-labeled Fe_3C in our reaction mixture (*6*). The specific activity of the nondiamond carbon in the mixture was of the order of 5.8×10^5 disintegrations per minute per gram; hence, the growth of as little as 10 micrograms of diamond could be readily detected by beta counting.

The Experiments

Cementite was prepared by fusing spectroscopically pure iron in a plumbago crucible and casting the melt into a graphite mold at 1550°C. The resulting alloy was found to contain 3 percent carbon—that is, 45 percent Fe_3C. A sample of this alloy was then reduced to shavings suitable for use in the pressure apparatus. An x-ray pattern taken of the shavings revealed only α-iron and Fe_3C. No impurities could be detected on spectrographic analysis, and no residue remained on dissolution of the shavings at room temperature with 6*N* hydrochloric acid.

Carbon-14 labeled cementite was prepared by fusing C^{14}-labeled graphite, spectrographic carbon, and electrolytic iron in a water-cooled copper arc furnace (*7*). X-ray examination of this sample (37 percent Fe_3C) showed the presence of only cementite and α-iron.

To prepare nickel carbide, weighed samples of NiO were reduced at 250°C by passing a stream of H_2 gas over the powder at a rate of 0.1 liter per hour until the loss in weight corresponded to that calculated by the stoichiometric reaction. The metal powder was then carburized at 250°C with CO gas (*8*)

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Table 1. X-ray pattern of insoluble fraction from high-pressure experiments compared with patterns of chromite (*18*) and diamond *d*, Spacing between lattice planes; *I*, intensity; *hkl*, Miller indices.

Observed			Chromite		Diamond	
d	*I**	*hkl*	*d*	*I*	*d*	*I**
4.7	*vw*	111	4.80	50		
3.20	*vw*					
2.90	*w*	220	2.93	60		
2.72	*w*					
2.49	*s*	311	2.499	100		
2.37	*vw*	222	2.390	*vf*†		
2.27	*vw*					
2.22	*vw*					
2.15	*vw*					
2.06	*m*	400	2.070	70	2.07	*vs*
1.58	*w*	511	1.592	90		
1.46	*m*	440	1.461	90		
		135	1.398	*vf*†		
		130	1.310	20		
1.25	*vw*	553, 622	1.261	50	1.26	*m*
1.20	*vw*	444	1.196	30		
		155, 117	1.158	30 20		
1.11	*vw*	642	1.107	30		
1.09	*vw*	731	1.079	60	1.075	*mw*
1.04	*vw*	800	1.035	30		
		110, 114	0.975	20		
		111, 157	0.956	40		
		120	0.927	30		
					0.890	*w*
		139	0.869	30		
		112	0.846	60		
					0.819	*m*
		134, 150	0.811	10		
		159, 377	0.801	40		

* In increasing order, the intensities are *vw, w, mw, m, s, vs* (very weak, weak, medium weak, medium, strong, very strong). † Very faint.

Table 2. Decomposition of Fe_3C in FeS melts at atmospheric pressure.

Run	Sample	Temperature (°C)	Time (min)	Count rate of residue (count/min)	Conversion to diamond (%)
A7	Iron-rich FeS, Fe_3C	1040 ±20	25		
A9	Iron-rich FeS, Fe_3C	1030 ± 20	25		
A12	Troilite, Fe_3C^{14}, diamond	1000 ± 25	25	0.02 ± 0.03	$\leqq$0.004
A14	Troilite, Fe_3C^{14}, diamond	1025 ± 25	15	0.10 ± 0.03	$\leqq$0.005

Table 3. Solid-state decomposition of Fe_3C at elevated pressure and temperature.

Experiment No.	Pressure (kilobar)	Temperature (°K)	Time (hr)	Count rate of residue (count/min)	Conversion to diamond (%)
P7	35 ± 2	823 ± 5	72		
P8	35 ± 2	873 ± 5	48		
P9	35 ± 2	923 ± 5	69		
P13	30 ± 2	565 ± 5	64		
P14	30 ± 2	650 ± 5	65		
P15	35 ± 2	730 ± 5	65		
P16	35 ± 2	773 ± 5	70		
P18	29 ± 2	733 ± 5	72		
P19	25 ± 2	733 ± 5	72		
P20	18 ± 2	733 ± 5	72		
P43	48 ± 2	648 ± 5	240	0.3 ± 0.1	$\leq$ 0.2
P43 (recycle)				0.03 ± 0.04	$\leqq$ 0.01

at a flow rate of 0.1 liter per hour. Nitrogen, at the same flow rate, was used during periods of cooling and heating to prevent carbonyl formation. The sample was periodically removed for weighing in order to determine the carbon content. After carburization for 281 hours, the increase in weight of the best sample corresponded to the increase to be expected with a carbon content of 4.50 percent or a Ni_3C content of 71 percent. X-ray examination of the resulting powder revealed only Ni_3C and Ni. No residue remained after dissolution of the powder in 6*N* HCl at room temperature.

The samples were mounted on aluminum plates (0.63 mm thick), covered with Mylar film (0.9 mg/cm^2), and counted in a Sugarman type β proportional counter with a gold-plated Mylar window (1.5 mg of gold per square centimeter). The counter was shielded with 5 centimeters of lead and 0.6 centimeter of aluminum. The samples were usually counted at 2 or 5 millimeters from the counter window.

Daily background counts were taken for several weeks before and after the sample counts. The variations were strictly statistical, and thus the background correction could be determined with high accuracy. In the two geometries used, the background counts were 6.04 ± 0.02 and 6.18 ± 0.03 counts per minute, respectively.

Absolute counting efficiencies were difficult to determine, in view of the highly nonuniform particle-size distribution of the samples. Owing to the very low energy of the C^{14} beta radiation, self-absorption effects were large even for particles of fractional-millimeter size. Conservative estimates of the counting efficiencies are 4 and 3 percent for the two geometries used. The percentage of recovery for any diamond present could not be determined directly but was estimated to be greater than 50.

The experiments at atmospheric pressure were conducted by fusing iron sulfide (both artificial and meteoritic) to which iron carbide (5 to 10 percent) had been added, at temperatures ranging from 975° to 1075°C for periods of 15 to 30 minutes and cooling to room temperature within a few minutes. Fusions were made in crucibles of various materials (Vycor, porcelain, recrystallized Alundum, and meteoritic iron), under fluxes of KCl, and in a vacuum. Decomposition of the carbide was virtually complete during this time. The extrapolated times for graphitization of diamond at these temperatures range from 1700 to 180 years (*9*, *10*) so there was no danger of graphitizing any diamond which might form. The cooled melt was then dissolved in HCl, and the residue was treated with $HClO_4$ to dissolve the graphite (*11*). Any solid material remaining was then x-rayed. We observed no lines attributable to diamond.

In several experiments, diamond seed and Fe_3C^{14} were added. In no such case did the final residue yield a count appreciably higher than the background level. The added C^{14} had counting rates of 2500 to 3000 counts per minute, whereas the observed counting rates were of the order of 0.1 ± 0.1 count per minute above the background level.

The pressure apparatus used in the experiments at elevated pressure was of the "simple squeezer" type (*12*). Cemented chromium carbide pistons were used exclusively. The powdered sample (usually 40 to 50 mg) was placed inside a washer sandwiched between two pieces of metal foil (Fig. 2). Initially, platinum-rhodium foil (Rh, 10 percent) 0.03 millimeter thick was used to contain the samples, but because this foil was difficult to dissolve, pure platinum foils were substituted. In order to preclude possible undesirable side reactions with platinum, a few duplicate experiments were performed with copper foil (Cu, 99.9 percent) or low-carbon steel. Since no difference in results was observed, pure platinum foil was used exclusively in the remainder of the experiments.

The washers were usually of case-hardened pure iron, although stainless steel washers were initially used. These latter were found to have a large chromium content which unnecessarily complicated the thermodynamic situation [ΔF°_{298} for the formation of $Cr_4C = -16.8$ kcal/mole (*5*)], and therefore the pure iron washers were substituted. A few experiments were run with pure copper washers, but these washers were not able to withstand the stress, and the sample extruded out. In many of the experiments we found that the sample, foil, and retaining washer were "welded" together and could not be separated. In these cases the entire assembly was dissolved.

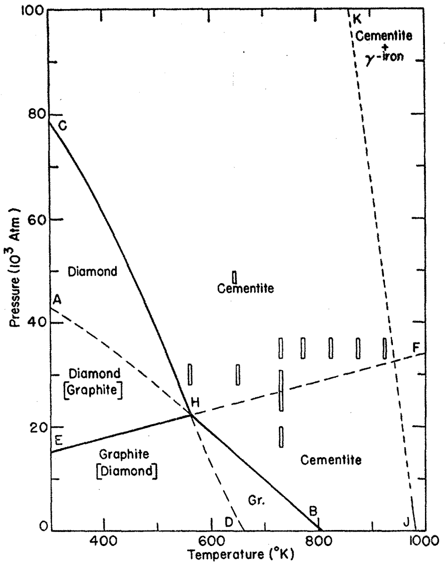

Fig. 1. Pressure-temperature conditions for Fe_3C experiments.

After the experiment had been completed, the sample was removed and partially dissolved in concentrated HCl at about 90°C. Enough concentrated HNO_3 was then added to dissolve the foil, and the heating was continued until the reaction was complete. The residue was centrifuged, washed, and dried. It was then heated for 6 hours in an atmosphere of Cl_2 at 450°C, washed, and separated into a light ($d \leq 2.9$) and a heavy ($d \geq 2.9$) fraction by means of bromoform.

Control experiments in which diamond seed (of 3- to 6-μ diameter) were used revealed the somewhat surprising fact that the diamond was quantitatively recovered in the light phase. Apparently the light phase consisted either of submicrocrystalline graphite growing on diamond centers or of an intimate mixture of graphite carbon and diamond. No crystalline material was detected in the heavy fraction.

The two fractions were then washed with acetone, mounted on the end of a Lusteroid rod coated with a solution of linseed oil (50 ml of oil per 100 ml of solution) in carbon tetrachloride, and x-rayed. Since the diffraction pattern of diamond would appear if the diamond were present at levels higher than about 4 to 5 percent, as little as 5 micrograms (corresponding to 5-percent conversion) could have been unambiguously detected.

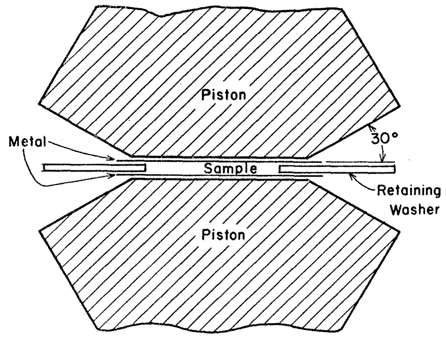

Fig. 2. Schematic arrangement of sample assembly in the pressure apparatus. [Courtesy *Am. J. Sci.*]

In some cases one or more diffraction lines were observed at spacings corresponding to those of diamond (Table 1). The two fractions of these runs were then further treated in one of the following ways.

Light fractions were heated for 1 hour with a solution of KNO_3 in concentrated H_2SO_4—a treatment which has been used by Wentorf (*13*) in oxidizing graphite while leaving diamond unaffected. In our early experiments, light fractions were treated with perchloric acid to destroy graphite (*10*, *11*). Although this treatment did not seem to affect polycrystalline meteoritic diamond lumps or diamond powder of particles 3 to 6 microns in diameter, the possibility remained that very finely powdered diamond of very small crystallite size might be attacked by the perchloric acid (*14*). Sarma and Mayeda (*15*) later observed this to be the case. For this reason, we avoided perchloric acid like the plague in later runs.

All heavy fractions of the Fe_3C runs (experiments P7 to P20) showed six to twelve unidentified lines in addition to three lines in the correct positions for the three diamond lines observable with FeKα radiation. This fact caused us to feel some elation, which was soon tempered by the discovery that all of these lines were due to chromite (*16*). Apparently, diffusion of Fe through the platinum foil was rapid enough to permit formation of chromite at the foil-piston interface. In subsequent x-ray work, CuKα radiation was used. With the latter source two additional spacings of diamond appear, which are not duplicated in the chromite pattern (*17*, *18*). When these lines were used as the criterion, no positive identification of diamond was made in any of the Fe_3C runs.

The heavy fractions of Ni_3C and Ni_3C–Fe_3C were found to contain a great deal of ignited chromic oxide, evidently an oxidation product of the chromium carbide piston. This oxide was found to be unaffected by fusion with $KHSO_4$ but was dissolved by evap-

Table 4. Solid-state decomposition of Ni_3C and Ni_3C–Fe_3C mixture at elevated pressure and temperature.

Experiment No.	Pressure (kilobar)	Temperature (°K)	Time (hr)	Count rate of residue (count/min)	Conversion to diamond (%)
P29	35 ± 2	823 ± 5	65		
P33	35 ± 2	950 ± 5	67		
P34	45 ± 2	743 ± 5	67		
P35	37 ± 4	958 ± 5	90		
P36	50 ± 2	648 ± 5	90		
P37	43 ± 2	848 ± 5	65		
P38	43 ± 2	843 ± 5	350		
P41	43 ± 2	843 ± 5	184	0.29 ± 0.03	≤ 0.1

oration with HF in the presence of CrO_3 (*19*). In these experiments x-ray examination revealed faint lines occurring at positions corresponding to the principal spacings of chromite. It is evident, therefore, that some chromite had formed, probably by reaction between the washer and piston, and that at least part had survived the chemical treatment described above.

Discussion

Decomposition of cementite melts at atmospheric pressure, in the absence of diamond seed, never revealed an x-ray pattern attributable to diamond (Table 2). In those cases where C^{14} had been added, the counting rate observed was not appreciably higher than the background level. It would thus appear that no diamond formed at atmospheric pressure through a metastable-phase mechanism. To the extent that these conditions were representative of those in the Canyon Diablo impact, the conclusion that the Canyon Diablo diamonds did not form as a metastable phase seems justified.

The experiments carried out at elevated pressure, with their results, are listed in Tables 3 and 4 and illustrated in Figs. 1 and 3. The rectangles shown in Figs. 1 and 3 represent experimental uncertainties in pressure and temperature. One of the experiments listed in Table 4 and illustrated in Fig. 3 was carried out with a mixture of Ni_3C (80 percent) and Fe_3C^{14} (20 percent). The thermodynamic behavior of this system should be similar to that of pure Ni_3C, and since the diffusion rate of the carbon is likely to be higher than the rate of precipitation, the C^{14} was expected to act as a tracer for the whole system. In no case did we obtain evidence of the growth or formation of diamond. True, a slight positive counting rate was observed in some of the tracer experiments (Nos. P41 and P43), but whenever these samples were recycled (experiment P43), the activity disappeared. Apparently, the activity was contained in traces of graphite carried through the procedure, and it seems likely that recycling of the remaining samples would have brought the activity down to background levels.

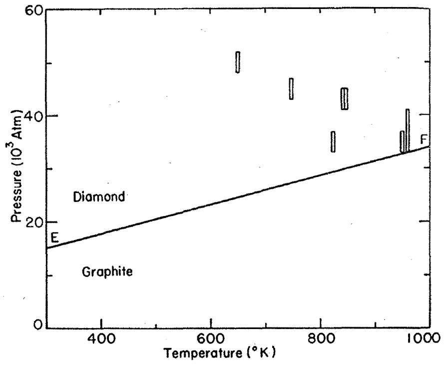

Fig. 3. Pressure-temperature conditions for Ni_3C experiments.

Similarly, none of the x-rayed samples showed evidence of diamond, once allowance was made for interference by chromite.

It now has to be shown that the negative results of the experiments listed in Tables 3 and 4 were not due simply to a low rate of carbide decomposition. That the carbide did indeed decompose is proved by the observation that all high-pressure runs yielded crystalline graphite, whereas samples of the original materials, Fe_3C and Ni_3C, treated by the same chemical procedures yielded no residue. Thus, the presence of appreciable amounts of graphite indicates that the carbide had decomposed to a significant degree (*20*). Consequently, the value given in the literature for the standard enthalpy of formation of cementite [$\Delta H^\circ_{298} = 5.0$ kcal/mole (*5*)] must be too low, as we conjectured in our previous report, and the lines *AB* and *CD* in Fig. 1 should be shifted to the right by a few hundred degrees. This shift expands the field in which decomposition of cementite can occur.

These experiments are gratifying inasmuch as they confirm the view, expressed in our previous report, that graphite can form as a metastable product above the graphite-diamond equilibrium line. They are disappointing inasmuch as we have been unable to channel this decomposition in the direction of the stable reaction product, diamond. Just why the metastable product graphite is favored even at high pressures is a matter of conjecture. Submicroscopic graphite nuclei were probably present in the original material in such large numbers as to favor the graphite path overwhelmingly. Moreover, it is conceivable that the added diamond crystals could not act as effective nucleation centers, owing to surface effects. Perhaps future experiments should be designed to take both possibilities into account.

Conclusions

In none of our experiments were we able to observe any evidence of diamond formation or growth. It thus appears unlikely that the metastable-phase mechanism was of importance in the formation of the Canyon Diablo diamonds. The discovery of coesite in the sandstone of Meteor Crater (*21*) and the successful laboratory conversion of graphite to diamond by explosive shock (*2*) seem to favor the mechanism of a direct conversion of graphite to diamond by the impact shock. Further, we found that the direct solid-state decomposition of cementite at pressures below 45 kilobars and temperatures of 650° to 1000°K does not yield diamond in times of about 1 week (*22*).

References and Notes

→ M. E. Lipschutz and E. Anders, *Geochim. et Cosmochim. Acta* **24**, 83 (1961).
2. P. S. DeCarli and J. C. Jamieson, *Science* **133**, 1821 (1961).
→ F. P. Bundy *et al.*, *Nature* **176**, 51 (1955);
→ H. P. Bovenkerk *et al.*, *ibid.* **184**, 1094 (1959).
4. H. Seltz, H. S. McDonald, C. Wells, *Am. Inst. Mining Met. Engrs. Tech. Publs. No. 1137* (1940); H. A. Schwartz, *Trans. Am. Soc. Metals* **23**, 126 (1935); F. D. Richardson, *J. Iron Steel Inst.* (*London*) **175**, 33 (1953).
5. *Natl.* Bur. *Standards* (*U.S.*) *Circ. No. 500* (1952).
6. We are indebted to Professor H. E. Suess for this suggestion.
7. We are indebted to Dr. D. Sunderman of the Battelle Memorial Institute for the preparation of this compound.
→ H. A. Bahr and T. Bahr, *Ber. deut. chem. Ges.* **61**, 2177 (192 → J. Schmidt, *Z. anorg. u. allgem. Chem.* **216**, 85 (193 → L. C. Browning and P. H. Emmett, *J. Am. Chem. Soc.* **74**, 1680 (1952).

9. P. Corriez, *Compt. rend.* **202**, 59 (1936).
10. F. S. Phinney, *Science* **120**, 393 (1954).
11. ———, *ibid.* **120**, 114 (1954).
12. D. T. Griggs and G. C. Kennedy, *Am. J. Sci.* **254**, 722 (1956).
13. R. H. Wentorf, Jr., personal communication (1960).
14. P. S. DeCarli, personal communication (1960).
→ D. V. N. Sarma and T. Mayeda, *Geochim. et Cosmochim. Acta* **22**, 169 (1961).
16. We are indebted to Miss T. Isaacs for indexing the pattern of this material.
17. Some allowance for the chromite-diamond coincidence should probably have been made in the work of Sarma and Mayeda (*15*). Their "spinel" pattern and the diamond pattern have several coincidences, and the "spinel" pattern agrees very closely with the chromite pattern reported by G. L. Clark and A. Ally (*18*).
18. G. L. Clark and A. Ally, *Am. Mineralogist* **17**, 66 (1932).
19. J. Weise, German patent No. DRP 134130 (1901).
→ R. H. Wentorf, Jr., and H. P. Bovenkerk [*Astrophys. J.* **134**, 995 (1961)] report, in contrast to our findings, that Fe_3C does not decompose at pressure of 50 kilobars and at temperatures between 500° and 1200°K. In our experience the decomposition of cementite over the temperature range covered by our experiments is quite noticeable in 2 days but nearly imperceptible in times of a few hours. Possibly differences in heating times were responsible for the discordance of our conclusions and those of Wentorf and Bovenkerk.
21. E. C. T. Chao, E. M. Shoemaker, B. M. Madsen, *Science* **132**, 220 (1960).
22. We thank Drs. J. Goldsmith and J. Jamieson of the department of geology of the University of Chicago for their advice, Dr. Goldsmith for the use of his pressure apparatus, and Mr. D. Northrup for valuable assistance in operating the pressure apparatus. One of us (M.E.L.) is indebted to the U.S. Rubber Company for a predoctoral fellowship. This research was supported in part by the National Science Foundation.

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Immunological Studies of Gastrin

Abstract. Hog gastrin prepared by the Gregory method stimulates antibody production in rabbits. Rabbit anti-hog gastrin serum which was reacted with hog, dog, and human gastrins in vitro caused reduction of their gastric stimulatory effect, indicating a biological cross reaction among these species.

The existence of the antral hormone gastrin was postulated by Edkins (*1*) in 1906, but Komarov (*2*) was the first to obtain an active substance from the protein fraction of antral mucosal extracts. Subsequently, other workers (*3, 4*) have obtained substances capable of stimulating gastric secretion from the protein fraction of mucosal extracts prepared by various methods.

We have produced rabbit antibodies to hog gastrin which inhibited the gastric secretory effect of hog, dog, and human gastrin.

Gastrin was prepared from hog antral mucosa by the method of Gregory and Tracy (*4*). The final extract was lyophilized, and approximately 1.0 g of the dry powder was obtained from 1 kg of mucosa. In addition to gastrin, this product contains a quantity of inert protein and inorganic salts. It promotes a flow of acid gastric juice when given by subcutaneous injection to dogs with a gastric fistula or Heidenhain pouch.

Table 1. Data of secretory inhibition experiments. Each value recorded represents the average of two or more determinations.

Dose	Output HCl (meq/3 hr)	Reduction (%)
Hog gastrin (50 mg)	1.55	
Hog gastrin (50 mg) + rabbit anti-hog gastrin serum (3 ml)	0.61	61
Hog gastrin (50 mg) + rabbit anti-hog gastrin serum (6 ml)	0.51	67
Hog gastrin (50 mg) + normal rabbit serum (3 ml)	1.29	17
Hog gastrin (50 mg) + rabbit anti-hog Group A substance serum (3 ml)	1.32	15
Dog gastrin (75 mg)	3.53	
Dog gastrin (75 mg) + rabbit anti-hog gastrin serum (3 ml)	0.90	75
Human gastrin (30 mg)	1.08	
Human gastrin (30 mg) + rabbit anti-hog gastrin serum (3 ml)	0.39	64
Human gastrin (30 mg)	1.92	
Human gastrin (30 mg) + normal rabbit serum (3 ml)	1.87	3
Histamine phosphate (1 mg)	1.93*	
Histamine phosphate (1 mg) + rabbit anti-hog gastrin serum (3 ml)	2.06*	

* Output in 90 minutes.

New Zealand rabbits (weight, approximately 2.5 kg each) were injected in the four foot pads and the lateral thoracic wall skin with 30 mg of hog gastrin incorporated in complete Freund's adjuvant. Four to six weeks later each rabbit received a booster injection (20 mg of gastrin dissolved in distilled water) subcutaneously in the interscapular region. One week later animals were bled by sterile intracardiac puncture, and the serum was harvested. With merthiolate added as a preservative, the serum was stored at −20°C until used. The anti-hog gastrin titer of this serum was determined by the qualitative preciptin method of Heidelberger and Kendall (*5*); the average titer was 1/5000 to 1/10,000. Higher titers could be obtained with subsequent booster injections.

Agar gel diffusion experiments were performed according to the method of Wilson and Pringle (*6*). Plates were prepared, allowed to stand in a moist chamber at 37°C for 7 days, and then read.

Biological inhibition tests were performed in the following manner. Solutions of hog, dog, and human gastrin preparations were mixed with rabbit antiserum to hog gastrin and allowed to stand overnight in the refrigerator. The mixtures were then injected subcutaneously into dogs with a Heidenhain pouch, and the secretory responses were compared with those obtained by injecting the same doses of gastrin alone. The volumes of solution injected were equal in all cases. A control injection of gastrin reacted with normal rabbit serum (Difco Laboratories) was included in all inhibition experiments.

Secretion in response to the subcutaneous injection of gastrin normally starts within 30 minutes after the injection and has practically ceased after 3 hours. Accordingly, gastric juice was collected over a 3-hour period after the injection, and the total output of hydrochloric acid during this period was determined by titration with $0.1N$ sodium hydroxide with phenol red indicator.

Table 1 presents the data of several secretory inhibition experiments. Each value recorded represents the average of two or more determinations with the gastrin preparations indicated.

Incubation of 50 mg of hog gastrin with 3 ml of rabbit antiserum to hog gastrin caused a 61-percent reduction in the acid secretion produced by 50 mg of hog gastrin alone, and the reduction was slightly greater when the hog gastrin was incubated with 6 ml of serum. In contrast with this, incubation of gastrin with normal rabbit serum produced only a 17-percent reduction in secretion. That this reduction represents a nonspecific action of rabbit serum is suggested by the similar reduction achieved by incubation of hog gastrin with rabbit anti-hog Group A substance serum (Ortho Corp.). Gastrin prepared from dog and human stomachs was also reacted with rabbit anti-hog gastrin serum, and the secretory response was reduced by 75 percent and 64 percent, respectively.

To exclude the possibility that the anti-gastrin serum was producing inhibition of secretion by some mechanism other than the inactivation of gastrin, a portion of serum was incubated with a solution of histamine, and the mixture was injected subcutaneously. Rabbit anti-hog gastrin serum failed to depress the acid secretion induced by histamine.