shown in Fig. 1. This confirms the experience of Gregory and Tracy that pure gastrin stimulates both the protein and bicarbonate output by the pancreas in doses which are also effective stimulants of gastric secretion. In these experiments no attempt was made to stimulate the pyloric pouch.

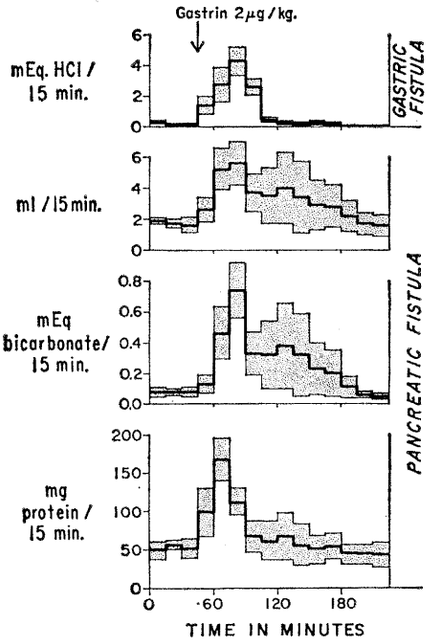

Fig. 1. Gastric and pancreatic responses to a single subcutaneous injection of gastrin I (2 μg/kg body weight). The gastric response is expressed as milliequivalents of hydrochloric acid secreted per 15 minutes; the pancreatic response is expressed in milliliters, milliequivalents of bicarbonate, and milligrams of protein, respectively, secreted per 15 minutes. Each value is the mean plus or minus the standard error of the mean of four experiments in two dogs.

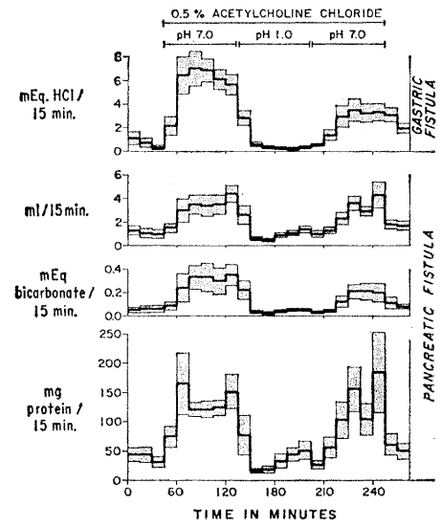

Fig. 2. Gastric and pancreatic responses to irrigation of the pouch of the pyloric gland area with 0.5 percent acetylcholine chloride (60 ml/hr) at *p*H 7.0 and *p*H 1.0. Each value is the mean of six experiments with three dogs.

Figure 2 shows the gastric and pancreatic secretory responses when the pouch of the pyloric gland area was irrigated continuously with a dilute solution of acetylcholine chloride. The irrigating fluid was maintained neutral until a response was evident; it was then acidified to *p*H 1.0, and then returned to *p*H 7.0. Stimulation of both the stomach and pancreas induced by acetylcholine in the pouch was easily inhibited by acidification. We have obtained similar, though smaller, gastric and pancreatic responses by irrigation of the pyloric pouch with a 5-percent solution of a peptone (Bactoprotone, Difco) and also with a 5-percent solution of liver extract. Again both responses were inhibited by acidification of the irrigating solution. Attempts to distend the pouch by means of a balloon were unsuccessful in terms of both gastric and pancreatic responses, possibly because of the mechanical difficulty of distending a pouch almost buried in the muscles of the abdominal wall.

Since the pyloric pouch in these experiments was transplanted and was devoid of any nervous connections with the gastrointestinal tract, the results indicate that a humoral agent, or agents, which excited both a gastric and pancreatic response was released by stimulation of the pouch. Although it is possible that acetylcholine or the protein hydrolysates were absorbed from the pouch and stimulated the stomach and pancreas directly, Robertson *et al.* (*7*) have shown conclusively that the gastric response to acetylcholine placed in a pyloric pouch cannot be attributed to absorption of the stimulant.

The demonstration that the same substances which are known to release gastrin from the pyloric gland area were effective stimulants of pancreatic secretion when introduced into the pouch supports the hypothesis that gastrin is also a pancreatic stimulant. The important observation that the effects were abolished by acidification of the antrum confirms this view; the release of gastrin from the pyloric gland area has been shown to be inhibited by acidification (*8*). If we had caused release of secretion from the pouch, it might have been expected that the pancreatic response would have been augmented rather than inhibited by acidification. The similarity between the gastric and pancreatic responses to both exogenous gastrin and to release of endogenous gastrin by stimulation of the pouch is further evidence for the hypothesis that gastrin is a physiological stimulant of pancreatic as well as gastric secretion.

R. M. PRESHAW
A. R. COOKE
M. I. GROSSMAN
Veterans Administration Center and *Departments of Physiology and Medicine, University of California, Los Angeles*

References and Notes

1. J. E. Thomas, *The External Secretion of the Pancreas* (Thomas, Springfield, Ill., 1950).
2. G. E. Drewyer and A. C. Ivy, *Proc. Soc. Exp. Biol. Med.* **278**, 186 (1929).
3. R. A. Gregory and H. J. Tracy, *Gut* **5**, 103 (1964).
4. E. L. Blair, D. G. Clark, A. A. Harper, H. J. Lake, T. Scratcherd, *J. Physiol.* **157**, 17P (1961).
5. R. M. Preshaw and M. I. Grossman, *Gastroenterology* **48**, 36 (1965).
6. Kindly supplied by Prof. R. A. Gregory and Dr. Hilda Tracy.
7. C. R. Robertson, K. Langlois, C. G. Martin, G. Slezak, M. I. Grossman, *Am. J. Physiol.* **163**, 27 (1950).
8. E. R. Woodward, C. Robertson, B. S. Fried, H. Schapiro, *Gastroenterology* **32**, 868 (1957).
9. Supported by NSF grant GB-667. R.M.P. is a Harkness Fellow of the Commonwealth Fund; A.R.C. is supported by a grant from the Post Graduate Committee of Medicine, University of Sydney, Australia.

1 March 1964

Response of the Rabbit Oviduct to a Tissue Adhesive

Abstract. *Sexual sterilization of mammals by the production of an obstruction at the tubo-uterine junction has been reevaluated experimentally. The application of 0.15 ml of methyl 2-cyanoacrylate monomere to the junction in rabbits produced, at intervals from 1 to 6 weeks later, histological, hydrodynamic, and functional evidence of tubal obstruction.*

Current techniques to produce permanent sterilization of the human female require abdominal surgery. A transcervical technique which is simple, safe, acceptable, and reliable would undoubtedly prove useful as a contraceptive method. The first known attempt to achieve this goal was reported in 1849, when a method was described which involved placing sufficient silver nitrate at the uterine cornu to produce scarring and cornual obstruction (*1*). In 1878 Kocks suggested the use of

electric cautery to produce cornual strictures, and since then a number of investigators have reported their experiences with this technique (*2*). A few workers claim permanent cornual obstruction, as determined by tests of oviduct patency in 80 to 90 percent of patients (*3*), but others have reported considerably less effective results (*4*).

We have reevaluated the cornual obstruction approach to sterilization. New Zealand white rabbits were used in the experiment. Potent chemical cauterizing agents such as phenol, trichloroacetic acid, and zinc chloride were introduced directly into the upper uterus and oviduct by means of a cannula passed through a small incision in the uterine wall. A number of different concentrations of each material were used; saturated phenol solution was the only material which produced significant tubal obstruction. Phenol was not studied further, however, because obstruction could not be produced consistently and the chemical was thought to be the cause of early postoperative deaths in three of the nine animals in which it was used.

The effect of a tissue adhesive, methyl 2-cyanoacrylate monomere (*5*), was also investigated. This material has been studied experimentally during the past 5 years for such purposes as joining blood vessels and gut, repairing the lung and trachea, closing the skin, fixing skin grafts, and producing hemostasis in liver and kidney wounds (*6*). The effect of the monomere on the reproductive organs, particularly on the oviduct, has not to our knowledge been described.

The monomere was administered with a fine-gauge polyethylene catheter insinuated through a 0.3-cm incision made in the uterine horn 2 cm caudad to the tubo-uterine junction. The tip of the tubing was placed by palpation at the junction and approximately 0.15 ml of monomere was ejected slowly. The catheter was maintained in position with as little pressure as possible for approximately 10 seconds, and gently withdrawn from the uterus. The uterine incisions were not closed.

Both tubo-uterine junctions of each of five rabbits were treated with monomere. There were no operative complications. The rabbits were killed at 1, 2, 3, 4, and 6 weeks after operation. After the death of a rabbit we removed the uterus and attempted to force physiological saline containing methylene blue through the uterine horns into the oviducts as a test of patency. Patency could not be demonstrated in any monomere-treated uterus but was uniformly demonstrated in the controls. An occlusive process occurs after ovulation at the tubo-uterine junction in the rabbit (*7*) but attempts to demonstrate this mechanism histologically have not been productive (*8*). We thought that this functional phenomenon did not interefere with the evaluation of patency in this study.

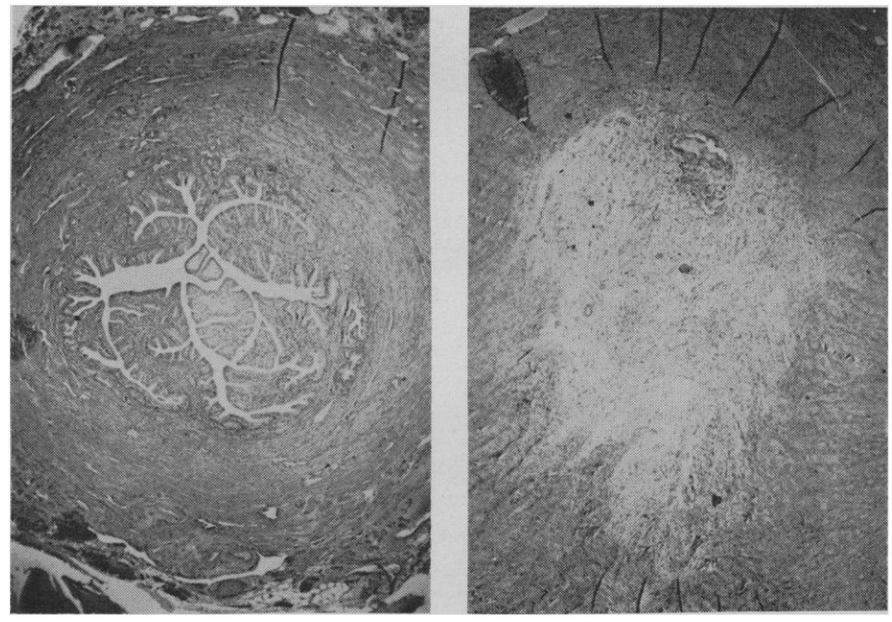

Fig. 1. Cross section of isthmic portion of rabbit oviduct. (Left) untreated; (right) 6 weeks after treatment with monomere.

The uteri were examined histologically by serial sections through the tubo-uterine junction and the oviduct. Sections of the untreated junction demonstrated a patent lumen (Fig. 1, left). In the obstructed areas of the rabbit killed 6 weeks after treatment with monomere (Fig. 1, right) the epithelium was destroyed and there was extensive fibrosis and foreign body giant cell reaction with numerous chronic inflammatory cells. Six weeks after treatment no large masses of the monomere could be identified, but a number of macrophages contained small particles of basophilic material thought to be the monomere. These tissue reactions to the monomere in the uterus are similar to those described in other tissues by previous investigators (*9*).

Six additional rabbits were operated on in the manner described. The uteri of two were treated with the monomere bilaterally, another two were treated bilaterally with normal saline instead of the monomere, and the last two animals were treated with monomere on one side and saline on the other. Beginning 1 week after surgery, these animals were bred at random to proven males. Within 1 month all of the six saline-treated uterine horns contained viable embryos. Only one of the six monomere-treated horns contained an embryo. This single embryo had implanted at the cervical end of a monomere-treated horn, adjacent to a saline-treated horn, probably because occlusion on the monomere-treated side was incomplete. It is also possible, but less likely, that a fertilized ovum from the contralateral side had migrated through the contralateral cervical canal, the vagina, and the cervical canal on the monomere-treated side.

Studies are now under way to determine if this method is applicable to other animal species. An instrument is being developed to be passed through the cervix of the primate uterus to make possible treatment of the cornual regions without laparotomy.

PHILIP A. CORFMAN
National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014
RALPH M. RICHART
HOWARD C. TAYLOR, JR.
Departments of Obstetrics and Gynecology and *Pathology, College of Physicians and Surgeons, Columbia University, New York*

References and Notes

1. R. Froriep, *Notizen aus dem Gebiete der Natur u. Heilkunde* **221**, 9 (1849).
2. J. Kocks, *Centralblatt Gynaekol.* **26**, 617 (1878); Y. V. Prudnikoff, thesis, Imperial Academy of Medicine of St. Petersburg (1912); R. L. Dickinson, *Surg. Gynecol. Obstet.* **23**, 203 (1916).
3. M. N. Hyams, *Am. J. Obstet. Gynecol.* **28**, 96 (1934); B. H. Sheares, *J. Obstet. Gynaecol. Brit. Commonwealth* **65**, 419 (1958).
4. L. A. DeVilbiss, *Am. J. Obstet. Gynecol.* **29**, 563 (1935); J. O. Porter, C. G. Sutherland, W. E. Brown, *ibid.* **74**, 341 (1957).
5. Provided by Ethicon, Inc., Somerville, N.J.
6. D. J. Hosbein and D. A. Blumenstock, *Surg. Gynecol. Obstet.* **118**, 112 (1964); D. A. Weilbaecher, F. J. Mathieu, I. Cohn, *Am. J. Surg.* **107**, 353 (1964); R. J. Wilder, H. Playforth, M. Bryant, M. M. Ravitch, *J. Thoracic Cardiovasc. Surg.* **46**, 576 (1963); H. H. Stone, *Am. Surgeon* **30**, 177 (1964); V. S. Wojnar, A. I. German, T. H. Moghul, D. Scarano, *Arch. Surg.* **89**, 237 (1964).
7. J. Brundin, *Acta Physiol. Scand.* **61**, 219 (1964).
8. G. S. Greenwald, *Fertility Sterility* **12**, 80 (1961).
9. W. C. Awe, W. Roberts, N. S. Braunwald, *Surgery* **54**, 322 (1963); B. Seidenberg, E. Garrow, R. Pimental, E. S. Hurwitt, *Ann. Surg.* **158**, 721 (1963).

10. Work performed in the departments of obstetrics and gynecology and pathology, College of Physicians and Surgeons, Columbia University, and at the Navy Medical Research Institute, Bethesda, Md. Supported in part by a grant from the Josiah Macy, Jr. Foundation.

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Hemoglobin and Oxygen: Affinities in Seven Species of Sciuridae

Abstract. *Studies of the respiratory function of the bloods of seven species of squirrels suggest that, in the evolution and adaptive radiation of this group, the oxygen affinity of hemoglobin has changed in a manner to better adapt the various species to different ways of life and different habitats. These changes are shown by the relative positions of the oxygen-dissociation curves of bloods of several species having dissimilar habits and environments.*

Rodents are the most numerous of mammals, in both number of individuals and number of species. Rodents are also old in evolution, having their origin in early Eocene. One well-defined family of rodents is the Sciuridae, or squirrels, which are almost worldwide in distribution. One of the interesting features in the natural history of squirrels is that they show considerable adaptive radiation. Some show burrowing habits, some are arboreal, some terrestrial; some can move by "gliding." Some species are solitary in habit, while others live in great colonies. Some are diurnal in habit while others are nocturnal. These features make the group an interesting one for physiological studies.

Seven species of squirrel became available during a study of rodents' blood: the common American marmot, *Marmota monax*; the antelope ground squirrel, *Amnospermophilus harasii*; the round-tailed ground squirrel, *Spermophilus tereticaudus*; the thirteen-striped ground squirrel, *S. tridecemlineatus*; the gray squirrel, *Sciurus carolinensis;* the prairie dog, *Cynomys ludovicianus*; and the flying squirrel, *Glaucomys volans*. Respiratory functions of blood of prairie dogs had been previously studied (*1*).

Blood samples were drawn by direct cardiac puncture; animals were tranquilized by small intraperitoneal injections of Nembutal, and minimal amounts of heparin were used as an anticoagulant. Blood samples were kept cold pending transfer to tonometers; all tonometric analyses were completed within 2 hours of blood withdrawal. In all instances mature adult animals in good physiological condition were used; no gross pathology was apparent at autopsy. An abbreviated description of the methods of blood equilibration (*1*) follows: Both oxygen and carbon dioxide were measured by use of 0.5-ml pipettes; the procedure used for these combined analyses has been described by Van Slyke and Neill (*2*). After blood was delivered to the Van Slyke apparatus, the remaining volume was extruded into a volumetric flask (50-ml) containing Drabkin's reagent. The pipette volume up to the lower mark was rinsed several times with this reagent; this solution was used for determination of total hemoglobin; the factor of 1.34 times the gram percentage of hemoglobin was used to give the total oxygen capacity of hemoglobin. Total oxygen capacity of a sample of blood was also determined by saturation of the blood with a pO_2 of 200 mm-Hg. Both methods employed for determination of oxygen capacity agreed within the errors of the analytical methods. Measurement of oxygen capacity on the same sample of blood as used in the Van Slyke analysis has obvious advantages.

Gas used in tonometric equilibration was delivered from large cylinders of mixed gases which had been analyzed in my laboratory. Gases were completely saturated with water vapor at the temperature of equilibration; this becomes very important when small samples of blood are equilibrated in open tonometers. Equilibrations of all samples were for 20 minutes at 37°C.

Table 1. Constituents of bloods of various species of squirrel. Numbers of specimens, followed by average body weights (g), appear in parentheses.

Hemoglobin (g/100 ml)	Hematocrit (% erythrocytes)	Erythrocytes (10^6-mm^3)
Marmot (4/2840)		
13.2	38.9	6.31
Gray squirrel (5/505)		
14.4	44.2	7.73
Prairie dog (9/1280)		
15.1	47.3	9.50
Round-tailed ground squirrel (5/282)		
12.6	40.5	7.31
Antelope ground squirrel (5/100)		
13.2	42.2	8.39
Thirteen-striped ground squirrel (3/153)		
14.9	45.8	10.67

Determinations of hemoglobin, hematocrit, and red blood count were also made. Hematocrit values were found by use of a microhematocrit centrifuge. Blood counts were made by the orthodox method of dilution and counting on a Neubauer slide. Hemoglobin values were determined with an Evelyn photocolorimeter; hemoglobin was read as metcyanhemoglobin.

Oxygen-dissociation curves of blood from seven species of squirrels are shown in Fig. 1; the curves are drawn through the average values obtained. No attempt was made to determine oxygen saturation below 15 percent or above 85 percent. Oxygen pressure to attain 50-percent saturation of the blood, where the ratio of hemoglobin to oxyhemoglobin was 1:1 at *p*H 7.40 and 37°C, had a standard deviation

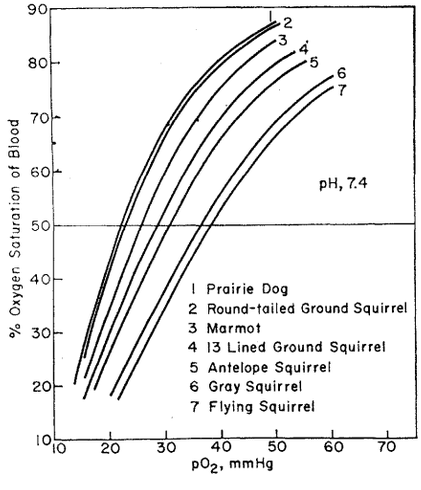

Fig. 1. Oxygen-dissociation curves of whole bloods of seven species of squirrel.