have clinical significance which is being evaluated currently. Other efforts are being directed toward isolation and identification of the diffusible substance and the plaque material which it produces and an elucidation of the specific substrates involved.

The diffusible substance that activates production of the red band differs from the substance that produces the plaque material. This is demonstrated by the fact that whereas all the bloods used for preparation of the media were able to act as substrate for the red band, a few did not act as substrate for production of plaques.

Summary. Macroscopically visible lipid plaques were produced on the surface of human blood agar plates and on 10% plasma agar plates when they were inoculated centrally with certain strains of coagulase-positive *S. aureus* or exposed to active sterile agar segments (ASA). Similar lipid plaques covered the surface of liquid media containing plasma when activated sterile agar segments were suspended in the media. Staphylococcus antitoxin prevented the appearance of the lipid plaques. The lipid plaques consist primarily of octadecenoic acid, both free and in triglycerides. Lesser amounts of stearic, palmitic and various shorter chain acids are also present. The most likely explanation for the phenomenon is that a lipase released by certain strains of staphylococci acts upon the lipids in human plasma.

1. Weld, J. T., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 693.

2. Hunt, G. A., Moses, A. J., *Science,* 1958, v128, 1574.

3. Metcalfe, L. D., Schmitz, A. A., *Anal. Chem.,* 1961, v33, 363.

4. West, E. S., Todd, W. R., *Textbook of Biochemistry,* 3rd ed., Macmillan, N. Y., 1961, p891.

5. Elek, S. D., *Staphylococcus Pyogenes,* E. S., Livingstone, Ltd., Edinburgh and London, 1959, p290.

6. Gillespie, W. A., Adler, V. G., *J. Path. Bact.,* 1952, v64, 187.

Received November 7, 1962. P.S.E.B.M., 1963, v112.

A Permanent Rat Gastric Fistula. (28074)

S. A. KOMAROV, S. P. BRALOW AND E. BOYD (Introduced by Harry Shay)
Fels Research Institute, Temple University School of Medicine, Philadelphia, Pa.

Our preliminary report(1) on basal gastric secretion in chronic fistula rats aroused much interest in our method of producing a permanent type of rumenal gastric fistula. We and others(2-4) believe that a chronic fistula in the rat may be of great value for investigation of basic problems of gastric secretion. It, therefore, seems desirable to describe in detail the technic used during the past 3 years.

Methods. A miniature stainless steel cannula of the type extensively used in Pavlov's laboratories was developed. The cannula is 10 to 12 mm in length with an inner bore of 3 mm diameter and a beveled flame at each end (Fig. 1). The inner bore at one flange is threaded to permit insertion of a removable screw which acts as a stopper between experiments. The lumen of the cannula permits a snug fit with a No. 8 French catheter.

Fasting prior to surgery was found to be unnecessary and a rumen containing food is easier to identify and to exteriorize. Under ether anesthesia, the left upper quadrant of the abdomen is shaved and the animal placed on its right side. An incision 5-10 mm in length is made about 5 mm below and parallel to the lower left costal margin between the parasternal and left mammary lines. This approach has been described(5). When the deep abdominal muscles are divided, 2 guide sutures are inserted at the margins of the muscular incision and held in place by small hemostats. The most dependent area of the rumenal wall is then drawn up through the incision. This area should be close to the greater curvature but far enough away from the glandular portion of the stomach to avoid sacrificing secretory mucosa. To permit ade-

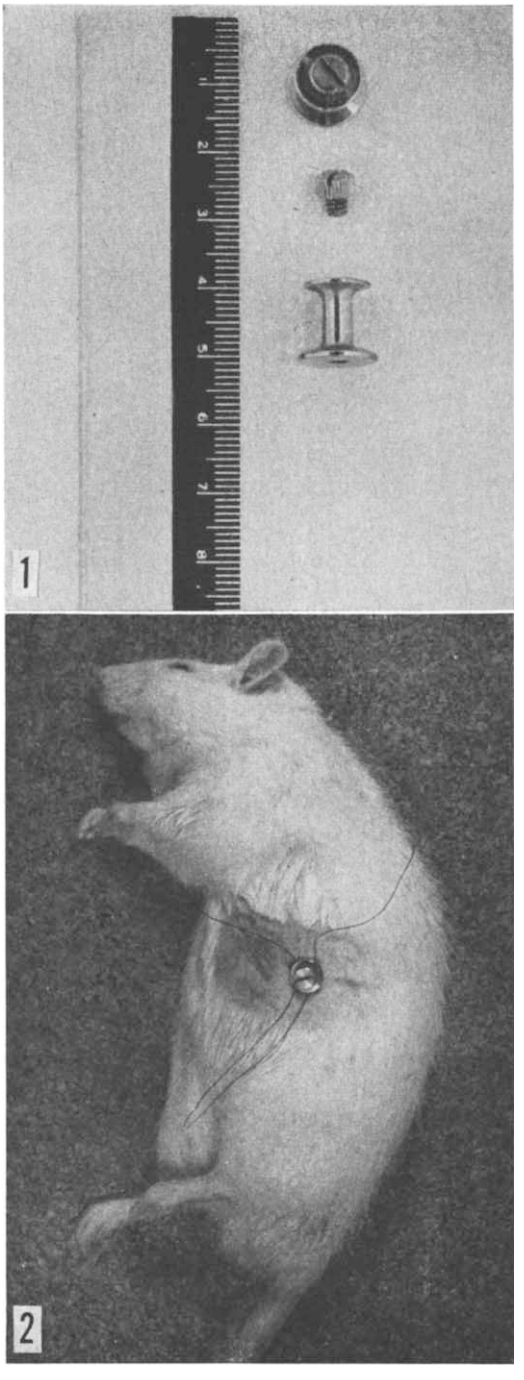

FIG. 1. A small Pavlov type of stainless steel cannula, 10-12 mm long, has a beveled flange at each end. The inner bore of one flange is threaded to permit insertion of a removable screw acting as a stopper between experiments.

FIG. 2. Cannula was sutured into the rumen through an incision made between parasternal and mammary lines about 5 mm below and parallel to lower left rib margin.

quate mobilization of the rumen, it may occasionally be necessary to ligate and section the ligament connecting the superior portion of the rumen to the under surface of the left diaphragm.

A small incision is now made in the exposed portion of the rumen and a purse string suture is loosely placed around the margin of the opening. The unthreaded flange of the cannula is inserted into the rumen and the suture is pulled tight around the shaft of the cannula and tied. It is advisable to wrap a piece of omentum around the shaft. The incision is then closed by 2 layers of interrupted sutures. The deep abdominal muscle is closed first by a suture at each side of the cannula, taking into each a bit of rumen to insure a tight fit. Guide sutures, previously laid down, can be used for this purpose. One or two more interrupted sutures through the abdominal muscles are occasionally required, and the incision is closed with skin sutures (Fig. 2).

Usually experiments are started after a postoperative period of 2 to 3 weeks to allow sufficient time for healing and development of fibrosis around the cannula. During this interval, the animals are conditioned to the experimental procedure by keeping them in a Bollman "restraining cage" for periods of several hours.

Food is withdrawn for 18 hours before any experiment, but water is given *ad libitum*. On the morning of an experiment, the animal is placed in a Bollman cage and the stopper screw removed from the cannula. If the initial drainage contains detritus or food particles, the stomach is gently lavaged once or twice with 2 to 3 ml of warm saline and drained. A 6 cm length of rubber catheter is inserted through the lumen of the cannula and fixed in place with rubber cement. Drainage then continues for 30 minutes or longer until the gastric contents are clear. Hourly collections into a graduated centrifuge tube are then started.

Results and discussion. For our experiments, we have used male Wistar strain rats from our colony, 6 months of age and about 300 g in weight. Such animals are fully grown and matured and do not significantly

increase in weight on a diet of Rockland Rat pellets and drinking water for the next 1-2 years. This permits an adequate period for chronic or repeated experiments without need to consider the influence of changes in body weight which can alter gastric secretion(6).

As a rule, animals with such fistulae remain in excellent condition without stomach ulceration, erosions of the abdominal wall or leakage of gastric contents. No more than one experiment per week was done on any animal to permit adequate recovery after prolonged fasting. During the past 3 years we have produced chronic fistulas suitable for experimental purposes in 166 animals. Three or more experiments were performed in each of 84 rats, and in 16 animals, between 20 and 40 experiments. Valuable information concerning the mechanisms of the interdigestive phase of gastric secretion, influence of some anesthetics and effect of insulin hypoglycemia and of gastrin on this secretion have been obtained with this type of preparation (1,7-9). Long range investigations with almost weekly studies following total adrenalectomy(10), thyroparathyroidectomy and radioactive I^{131} have also been done.

Summary. A simple technic for preparation of a chronic rumenal fistula in the rat using a specially designed Pavlov's type of cannula is described.

1. Komarov, S. A., Bralow, S. P., *Physiologist,* 1960, v3, 96.

2. Lane, A., Ivy, A. C., Ivy, E. K., *Am. J. Physiol.,* 1957, v191, 221.

3. ———, *ibid.,* 1957, v191, 262.

4. Lin, T. M., Alphin, R. S., *ibid.,* 1958, v192, 23.

5. Komarov, S. A., Shay, H., Rayport, M., Fels, S. S., *Gastroent.,* 1944, v3, 406.

6. Shay, H., Sun, D. C. H., Gruenstein, M., *ibid.,* 1954, v26, 906.

7. Bralow, S. P., Komarov, S. A., Shay, H., *Fed. Proc.,* 1961, v20, 250.

8. Komarov, S. A., Bralow, S. P., Shay, H., Boyd, E., *ibid.,* 1962, v21, 263.

9. Komarov, S. A., XXII International Congress, Internat. Union of Physiol. Sciences, Leiden, 1962.

10. Shay, H., Bralow, S. P., Komarov, S. A., Kessler, W. B., *Fed. Proc.,* 1962, v21, 265.

Received November 8, 1962. P.S.E.B.M., 1963, v112.

Does "Restricted Diffusion" Occur in Muscle Capillaries?* (28075)

CHRISTIAN CRONE (Introduced by G. Asboe-Hansen)
Institute of Medical Physiology, University of Copenhagen, Denmark

The concept "restricted diffusion" implies a deviation of the ratio between the permeability coefficients from the ratio between the free diffusion coefficients. The occurrence of "restricted diffusion" is usually taken as evidence of the existence of pores in a membrane. Pappenheimer(7) took the finding as evidence for existence of pores in the capillary membrane of the hind limb of the cat. His ingenious method of determining the permeability coefficients involved application of van't Hoff's law: $\Delta\pi = RT\Delta c$, where $\Delta\pi$ is the osmotic pressure difference across the membrane, Δc is the difference in concentration, R is the gas constant and T is the absolute temperature. As the capillary membrane is permeable to both solutes and solvent and thus does not behave ideally, the introduction of a correction factor, σ, the reflection coefficient of Staverman(9), becomes important. Lack of knowledge of the magnitude of the correction factor (which, of course, varies for molecules of different sizes) may disturb the calculation of a true permeability coefficient. This point of view was put forward by Ussing(10) and by Grim(4) and later by Kedem and Katchalsky(5).

The criticism advanced on theoretical grounds has not been tested experimentally. It is, however, possible to calculate the permeability coefficients from data obtained by use of another experimental principle, the

* Supported by a grant from P. Carl Petersen's Foundation, Copenhagen.