8. Tattersall, R. N., Seville, R., *Quart. J. Med.*, 1950, v19, 151.

9. Lansing, A. I., Ciba Foundation Colloquia on Aging, J. and A. Churchill, London, 1955, v1, 88.

10. Kao, K. Y. T., Boucek, R. J., Noble, N. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 535.

11. Conway, E. J., *Microdiffusion analysis and volumetric error*, Van Nostrand Co., 1950, 3rd edit., 124.

12. Neuman, R. E., Logan, M. A., *J. Biol. Chem.*, 1950, v184, 299.

13. ———, *ibid.*, 1950, v186, 549.

Received March 5, 1959. P.S.E.B.M., 1959, v101.

Nature and Source of Appendical Secretion.* (24865)

JOHN F. PERRY, JR., MERLE K. LOKEN AND O. H. WANGENSTEEN
Dept. of Surgery, University of Minnesota, Minneapolis

Demonstration that the vermiform appendix of man(1), chimpanzee(2), and rabbit (3) secretes fluid at pressures approaching systolic blood pressure has provided a basis for understanding the pathogenesis of obstructive appendicitis in man. However, because of the simplicity of treatment of appendicitis by surgery, there has been little stimulus for study of the site or mechanism of this fluid production. The following experiments were undertaken to determine the cellular elements responsible for this process in the vermiform appendix. Normally the rabbit appendix secretes from 10 to 56 ml of a slightly cloudy, colorless liquid of specific gravity 1.001-1.006 and pH 8.1-8.5 in 6 hour period.

Method. Adult albino rabbits of both sexes were utilized. Measurement was made of volume of secretion and secretory pressure with appendix ligated at base and attached to a collecting balloon or manometer respectively. Several experiments were designed to modify or nullify participation by certain cellular elements: I. *Suppression of lymphoid elements.* a) Exposure doses of x-ray in range of 250-1000 R were delivered to a series of appendixes.† Volume and pressure measurements were made 2 to 21 days thereafter. b) Cortisone was given parenterally in dosage of 5 mg/day for 7 and 14 days and 12.5 mg/day for 14 days. This steroid causes dissolution of lymphocytes and actual decrease in weight of lymphoid elements(4). II. *Stimulation and exhaustion of mucous cells.* a) Pilocarpine(5) in 2.5 mg dosage was administered hourly for 4 hours prior to fluid collection and in another group of animals the drug in same dosage was given hourly during a 6-hour collection period. b) Mustard oil(6) in olive oil was instilled into appendical lumen for 6 hours and fluid collection and pressure measurements carried out thereafter. III. *Destruction of surface epithelium.* a) Silver nitrate in 2 and 5% concentrations was instilled into the appendix for 5 minutes prior to pressure and volume measurement. IV. To determine if a cellular process requiring oxygen utilization was actually involved, sodium cyanide M/600 in 2 ml amounts was placed in appendical lumen and pressure determined(7). Appropriate control volume and pressure measurements were made. Histologic examination was made of all appendixes studied. Specimens were fixed in formalin and stained with hematoxylin and eosin or fixed in absolute alcohol and stained by Mayer's mucicarmine technic.

Results. Volumes of fluid secreted by the appendix under the experimental conditions are shown in Table I. Following X-irradiation, there was significant decrease in volume of fluid produced, both in early post-irradiation period and after 3 weeks. Sections of irradiated appendixes uniformly demonstrated severe suppression of lymphoid elements; those appendixes showing great reduction in secretory volume uniformly demonstrated epithelial damage as well. Irradiation was quite

* Supported by the United States Public Health Service Grant RC-4780 (C2) and the Donald J. Cowling Fund for Surgical Research.

† For irradiation a G. E. Maximan x-ray machine was operated at 140 KV, 15 MA with no added filter (H.V.L. = 3.7 mmAl). Using 27 cm F.S.O. exposure dose rate was 232 roentgens/minute as measured with Victoreen r-meter.

TABLE I. Effect of Several Agents on Secretion by Rabbit Appendix.

No. of animals	Agent administered	Time from treatment to fluid collection	Collection period (hr)	Vol of section (ml) Mean	Range
6	None (control)	—	48	122	95-138
7	X-irradiation (1000 r)	21 days	48	36	10- 52
5	*Idem*	48 hr	48	45	28- 82
10	X-irradiation (250-750 r)	72 "	48	72	27-200
9	None (control)		6	23	10- 56
5	Cortisone, 5 mg/day, 7 days		6	5	3- 15
6	*Idem* 14		6	13	8- 25
7	Cortisone, 12.5 mg/day "		6	11	6- 20
5	Pilocarpine, 2.5 mg/30 min. for 8 doses (before collection)		6	21	11- 29
6	Pilocarpine, 2.5 mg/30 min. for 12 doses (during collection)		6	13	10- 23
5	Mustard oil (4 drops/25 ml olive oil) for 6 hr		6	24	13- 33
5	Mustard oil (15 drops/25 ml olive oil) for 6 hr		6	18	5- 26
4	Mustard oil (25 drops/25 ml olive oil) for 6 hr		6	26	15- 40
6	$AgNO_3$, 2% for 5 min.		6	15	10- 30
4	*Idem* 5%		6	11	3- 20

uniformly followed by increased number of mucous secreting goblet cells both of surface epithelium and crypt walls.

Administration of cortisone was followed by reduction in thickness of lymphoid layers to approximately one-half to one-third of normal with dissolution of considerable numbers of lymphocytes. Although there was decreased volume of fluid following cortisone administration, the decrease is significant only when the drug was given in 5 mg amounts daily for one week.

All animals given pilocarpine had extreme salivation and repeated defecation during period of administration. There is no significant difference in volume of fluid produced compared with control animals whether the drug was given before or during collection period. Mucicarmine stained sections of most appendixes showed no histologically demonstrable effect of pilocarpine on mucous cells. However, appendixes of 2 animals receiving the drug before, and of 3 rabbits receiving pilocarpine during fluid collection, showed definite increase in mucus production by goblet cells of crypt walls. This greater secretory activity by mucous cells was not associated with increased volume of fluid secretion in any of these animals.

The mucous membrane of the appendix was exposed to irritant effects of mustard oil in 3 concentrations. Erosion of surface epithelium and edema and hemorrhage of subepithelial tissues occurred in a few specimens so treated. Exhaustion of goblet cells of surface epithelium occurred in a few, but the majority of appendixes showed no erosion, edema or hemorrhage, and normal to increased amounts of mucus being produced by goblet cells of surface and crypt walls. In none, including those specimens showing hypersecretion by goblet cells, was there significant change in volume of secretion. Significant decrease in fluid production followed instillation of 5% $AgNO_3$ into the appendix, but not following use of this substance in 2% concentration. Organs so treated demonstrated fairly uniform damage to surface epithelium. In several specimens, coagulation necrosis of surface epithelium led to obliteration of crypt orifices and, presumably, as a result of continuing secretion, crypts were converted into cystic structures with complete pressure obliteration of the papillae.

Following application of NaSCN, no change from baseline intraluminal pressure occurred during 5 hours. In all other groups in which intraluminal pressure was determined, values

of 50 to 100 cm of water pressure were observed. These pressures are of the same magnitude as those obtained with control animals.

Discussion. Although total destruction of lymphoid elements of the appendix was not observed in these studies, suppression of these elements *per se* did not appear to modify appendical secretion significantly. Rather, decrease in secretion appeared to be related more to damage of epithelial elements than of lymphoid follicles.

The goblet cell apparently is not responsible for the large volume of secretion occurring in rabbit appendix. Stimulation of goblet cells was produced in several experiments with pilocarpine and mustard oil as evidenced in mucicarmine stained sections. In none of these animals was there an increased volume of secretion. Irradiation led to increase in numbers of goblet containing cells of surface epithelium and crypt walls, but to decreased rather than increased secretion.

Obliteration of papillae with conversion of crypts into globular cyst-like spaces following closure of crypt orifices suggests that secretion occurs at least in part in these crypts. Since cells of crypt walls appear to be predominately mucous cells, and since these do not appear to contribute significantly to the secretory process, epithelial cells overlying the papillae must be responsible. The role of surface epithelium and subepithelial elements was not clarified in these experiments. However, as it was possible to destroy surface epithelium by silver nitrate and have secretion continue, the epithelial elements of the papillae must contribute significantly to the process.

Summary. 1. Large volumes of fluid produced by vermiform appendix of the rabbit, result from a cellular process requiring oxygen utilization. 2. Suppression of lymphoid elements of the appendical wall produces no significant decrease in secretory volume or pressure. 3. Stimulation of mucous cells is not associated with augmented secretion. 4. Destruction of surface epithelium reduces but does not abolish secretion. It would appear that secretion stems primarily from epithelial elements overlying papillae in crypts.

1. Wangensteen, O. H., Dennis, C., *Ann. Surg.* 1939, v110, 629.

2. ———, *S.G.O.*, 1940, v70, 799.

3. Dennis, C., Buirge, R. E., Varco, R. L., Wangensteen, O. H., *Arch. Surg.*, 1940, v40, 929.

4. Dougherty, T. F., White, A., *J. Lab. and Clin. Med.*, 1947, v32, 584.

5. Florey, H., *Brit. J. Exp. Path.*, 1930, v11, 348.

6. Florey, H., Webb, R. A., *ibid.*, 1931, v12, 286.

7. Florey, H., *ibid.*, 1931, v12, 301.

Received March 9, 1959. P.S.E.B.M., 1959, v101.

ACTH *in vitro* on Release of Nonesterified Fatty Acids from Adipose Tissues of Adrenalectomized Rats. (24866)

MICHAEL C. SCHOTZ, G. M. C. MASSON AND I. H. PAGE
Cleveland Clinic Fn. and Frank E. Bunts Educational Inst.

The role of hormones in regulation of fat mobilization is not clear. During fasting plasma nonesterified fatty acids (NEFA) are released from fat depots and are transported to other tissues(1). It has been shown that factors affecting concentration of NEFA in plasma similarly affect their release from adipose tissue incubated *in vitro*(2,3). Addition of ACTH to incubating medium enhances release of NEFA from adipose tissue(4). Injection of this hormone into animals causes an increase in hepatic lipids which is prevented by adrenalectomy(5,6,7,8). Presumably, increased hepatic lipid is due to higher rates of mobilization of fat from adipose tissue(9). Thus, it is possible that the augmented level of liver lipids found in ACTH treated animals is due partially to increased release of NEFA from adipose tissue and transport of these fatty acids to the liver where they are esterified(10). The purpose of this investigation was to determine whether in rats, previous adrenalectomy had an effect on release of NEFA by epididymal adipose tissue, on incu-