many enzymes. The present data can be explained in two ways, first by postulating a simple catalysis of heat inactivation by hydrogen and hydroxyl ions and second by assuming that while Phage denaturation with corresponding loss of activity occurs at all pH values tested the reversal of denaturation and restoration of activity proceeds best within the pH zone close to neutrality. It is not possible to determine from the present experimental data which mechanism is actually concerned.

8235 C

V. Lymphatic Absorption in Simple Obstruction: Significance of Distention upon Its Occurrence.*

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It is generally conceded that the content of the obstructed bowel is toxic as is also that of the unobstructed gut. The matter of abnormal absorption from the obstructed bowel is still the subject of considerable debate and speculation. The possible avenues through which toxic material may be absorbed from the bowel are: (1) the mesenteric veins, (2) the lymphatics, and (3) transperitoneally by diffusion through the bowel wall. It has been well established by many investigators that under conditions of obstruction, venous absorption, at any rate for substances absorbed from the normal bowel, is decreased. Transperitoneal absorption apparently does not occur unless there is gross damage to the bowel wall by distention with impairment of its viability.[1]

In this study an attempt has been made to evaluate the occurrence of lymphatic absorption under conditions of simple intestinal obstruction and increased intra-enteric pressure. The absorption of dyes and of bacteria from the obstructed bowel was examined in the following manner:

Method and Results. Simple ileal obstruction was produced in 8 cats, and 20 cc. of 1% gentian violet or trypan blue was injected into

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[1] Scott, H. G., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 287; Sperling, Louis, and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**,

the bowel above the site of obstruction. Such animals succumbed after 24-120 hours. At autopsy, 3 showed colored lymph nodes. Closed ileal loops were prepared in 12 cats, and the loops were distended with dye until the walls became tense (marked increase of intra-enteric pressure). Ten of the 12 cats at autopsy (24-72 hours) showed blue nodes. In several of these cats as the dye was being injected under pressure into the lumen of the bowel, the dye could be seen after 5 to 10 minutes to outline Peyer's patches in the wall and later the lymph vessels in the wall; finally, with continued pressure the lymph vessels of the mesentery to the regional lymph nodes were visualized. This procedure was repeated *in vitro* with fresh excised viable specimens of normal and obstructed cat bowel with the following results: Pressures of 100 mm. of mercury in 2 normal loops forced the dye out into the lymphatics in 10 minutes. In another normal loop, a pressure of 50 mm. of mercury forced dye into the lymph vessels in one hour. When a fresh viable loop excised from a cat with simple ileal obstruction of 48 hours was subjected to 40 mm. pressure, the dye appeared in the lymph vessels in 30 minutes. When non-viable loops were subjected to increased intra-enteric pressure, the dye in addition to being forced rapidly into the lymphatics permeated directly through the bowel wall.

Fifteen normal control cats received varying amounts of dye by stomach tube and were operated upon at intervals of 24 and 72 hours. In none was the dye grossly evident in the mesenteric lymph nodes. Kagan recently has shown that the oral administration of trypan blue cut down the amount absorbed. When cats with simple ileal obstruction were given the dye by stomach tube, the dye did not appear in the lymphatics. Another control series (6 cats) was submitted to laparotomy, and a chemical peritonitis was induced with the intraperitoneal introduction of 5 cc. of tincture of iodine (to cause more profound intestinal stasis) and then the dye was administered by a stomach tube. At autopsy (24 to 72 hours) only one animal showed colored nodes. A third series of controls (5 cats) received the dye by enteric injection. The animals were operated upon again at intervals of 2, 5, 18, and 48 hours. In no case were the regional lymph nodes stained. At 5 hours, the dye had traveled from upper jejunum down into the colon.

The absorption of bacteria through the lymphatics was studied in the following manner: A heavy suspension of *B. pyocyaneus* was injected into the ileum of 6 normal cats. Preliminary control biopsy cultures of the regional lymph nodes made in several instances were negative. Cultures taken from the node one hour after intra-enteric

injection were positive in 2 cases and negative in 4 for *B. pyocyaneus;* in 2 of the latter, positive cultures were obtained when the animals were reoperated upon after 24 hours. In 2 additional cats in whom the bacterial suspension was introduced into closed loops under pressure for one hour, the cultures from the regional nodes proved positive.

Similar experiments were carried out in normal and obstructed dogs. Positive cultures were obtained from the regional lymph nodes in 2 of 9 experiments on normal dogs, and in all of 6 experiments performed on dogs with low ileal obstruction of 24 to 96 hours' duration. Control cultures of the lymph nodes taken before injection of the bacterial suspension were negative in every case. Cultures of the thoracic duct lymph flow in these experiments on 5 normal and 6 obstructed dogs proved entirely negative. Cultures of femoral and portal (loop) blood in these cases were all negative.

In the experiments *in vivo,* the positive nodes of the closed loop were stained a definitely deeper color than those of the simple ileal obstruction group. The percentage of positive nodes was also much higher in the closed loop group than in the simple ileal obstructions. This was probably due to the fact that a higher intra-enteric pressure is developed in the closed loop.

In the control experiment, the absorption of dye into the lymphatics is no doubt prevented by its rapid transport through the bowel. Transport, of course, is abolished in obstruction. In its stead there is stasis, distention and increased intra-enteric pressure. From these experiments, we believe that there is a high-grade correlation between obstruction and the grade of intra-enteric pressure present, and the occurrence of lymphatic absorption. These findings are in accord with those of Costain,[2] Murphy and Brooks,[3] and Stone and Firor,[4] who have stressed the lymphatics as an avenue of absorption in obstruction. Williamson and Brown[5] had previously been unable to demonstrate in the lymph of the thoracic duct the presence of *B. prodigiosis* previously introduced into the unobstructed bowel. Ogilvie,[6] however, found that hemolytic streptococcus may be absorbed into the lymphatics and be cultured from the lymph. Hibbard[7] in this laboratory collected lymph from the thoracic duct after

[2] Costain, W. A., *Surg., Gynec. and Obst.*, 1924, **38**, 252.

[3] Murphy, F. F., and Brooks, B., *Arch. Int. Med.*, 1915, **15**, 392.

[4] Stone, H. B., and Firor, W. M., *Tr. South. S. A.*, 1924, **37**, 173.

[5] Williamson, C. S., and Brown, E. I., *Am. J. M. Sc.*, 1923, **165**, 480.

[6] Ogilvie, W. H., *Brit. J. Surg.*, 1924, **12**, 752.

[7] Hibbard, J. S., personal communication.

injecting 2% ammonium hydroxide with ammonia gas into a loop of jejunum. The ammonia content of the lymph was increased 4 times when the intra-enteric pressure was raised to 60 mm. of mercury. In 4 cats the lymphatic pedicle at the base of the mesentery was ligated without prolongation of the survival period attending the establishment of simple ileal obstruction.

It would appear that the regional lymph nodes normally serve as an effective barrier against overwhelming absorption of bacteria into the blood stream via the mesenteric lymphatic channels. A true bacteremia probably does not occur and most likely is not the cause of death in uncomplicated simple intestinal obstruction as we were unable to obtain positive cultures of the test organism from the thoracic duct. The lymph nodes yielding positive cultures acted as efficient barriers and precluded more centripetal invasion. A toxemia of bacterial origin, however, has not been wholly excluded.

Conclusion. Intestinal obstruction and increased intra-enteric pressure are conducive to increased lymphatic absorption of dyes and bacteria into the regional lymph nodes. No evidence, however, was adduced to indicate that this occurrence is of great significance in the lethal issue of bowel obstruction.

8236 C

An Improved Method for Determination of Blood Carotene.*

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Following closely upon the rapid accumulation of our knowledge regarding the relation of plant carotene to vitamin A, increasing interest has been manifested by investigators in the study of carotenoids. Thus far the investigations have been concerned chiefly with the carotene content of human and animal organism under different physiological and pathological conditions. The need of a suitable method for determining small amounts of carotene in the circulating blood has become quite apparent.

The methods hitherto employed[1] are more or less unsatisfactory

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1 Van den Bergh, H., Muller, P., and Broekmayer, J., *Biochem. Z.*, 1920, **108**, 279; Connor, Ch. L., *J. Biol. Chem.*, 1928, **77**, 616; Van Eekelen, M., *Acta brevia*