potassium and phosphorus, the ratio of the average decrease being 3:2:1 respectively. This same sequence was found in the majority of cases, although in some, all 3 constituents decreased in the same proportion, due possibly to dilution of the muscle elements as a result of pathological change. This is not the rule, however, and if it may be assumed that in resting muscle creatine exists as the dipotassium salt of phosphocreatine, this would help to explain the ratios found in the loss in concentration since they correspond roughly with the intramolecular ratios found in this hypothetical compound. The loss of potassium and phosphorus is somewhat greater than that required by this hypothesis, but it is to be expected since the amount of creatine is quite insufficient to combine with all the potassium and phosphorus present in heart muscle.

9016 C

Effect of Oxygen on *Bacterium necrophorum* in the Isolated Colon Segment of a Dog.

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Oxygen has been employed in the experimental treatment of ulcerative colitis.[1, 2] In view of the almost constant association of *Bact. necrophorum* with this disease,[3, 4, 5] and the sensitivity of this organism to oxygen,[6] it seemed advisable to study the effect of oxygen on cultures introduced into the colon.

For these experiments a dog was used which had been operated upon and an isolated colonic segment prepared. This loop included about 7-8 cm. of terminal ileum, the open end of which was brought out through a stab-wound in the abdomen, providing an entrance to the colonic segment. The loop consisted of cæcum, the ascending and one-half of the transverse colon. The transverse colon was divided, the proximal opening closed with a purse string suture, and the distal open end anastomosed end to side to the small bowel to reestablish the flow of faecal current. The only exit of material

1 Felsen, Joseph, *Arch. Int. Med.*, 1931, **48**, 786.

2 Golob, Meyer, *J. Am. Med. Assn.*, 1936, **106**, 1725.

3 Dack, G. M., Heinz, T. E., and Dragstedt, L. R., *Arch. Surg.*, 1935, **31**, 225.

4 Dack, G. M., Dragstedt, L. R., and Heinz, T. E., *J. A. M. A.*, 1936, **106**, 7.

5 Dack, G. M., Dragstedt, L. R., and Heinz, T. E. In press.

6 Beveridge, *J. Path.*, 1934, **38**, 467.

from this loop of colon was through the stump of ileum which went to the outside. We have found this type of bowel-segment to accommodate considerable amounts of fluid without appreciable leakage. Material was introduced and withdrawn from the bowel by introducing a sterile, soft rubber urethral catheter into the bowel.

The following general technic was used for the whole experiment. A strain (101) of *Bact. necrophorum* was used which had been isolated at proctoscopic examination from the colon of a patient with severe ulcerative colitis. The cultures used in the experiments were grown in deep tubes of Rosenow's glucose-brain medium for 24 hours at 37°C. The supernatant fluid from these cultures was centrifugalized to concentrate the organisms. Part of the clear, centrifugalized, supernatant was discarded and the sediment suspended in the remaining portion, making a total volume of 40-50 cc.

The culture thus prepared was introduced into the colon. At intervals of 30 minutes, 1, 2, 4, 8, 12, etc., hours, specimens were removed. Decimal dilutions of from 1-10 to 1-1 billion were made in 9 cc. blanks of cystine (0.05%), dextrose (1%), veal-infusion broth. This diluting fluid was used because it does not appear to impair the viability of *Bact. necrophorum.* One cc. of the various dilutions was then pipetted into tubes of Rosenow's glucose-brain medium. The tubes were incubated at 37° C. and blood agar plates streaked from those showing turbidity and gas in 24 hours. The inoculated plates were incubated in anaerobic jars as previously described,[4] and examined after 48 hours' incubation at 37°C. *Bact. necrophorum,* if present in the dilutions cultured, was recognized by the green zone of hemolysis surrounding the colonies. This green zone developed several minutes after removal of the plate from the anaerobic jar. Confirmation of these colonies as *Bact. necrophorum* was established by microscopic examination and finding long filamentous, pleomorphic, granular forms. Characteristic colonies were picked and put back in Rosenow's medium to find out whether or not the growth and morphology remained typical. All cultures were tested on aerobic blood-agar slants to make sure that the recovered strains were true anaerobes.

By the use of this technic the relative concentration of *Bact. necrophorum* could be determined and any marked variation in their number thus easily observed. In all cases, before the suspension was introduced into the colon, the number of viable organisms was determined by the method previously described.

A determination was made of the rate of disappearance of *Bact. necrophorum* from the normal colon. This was done by introducing

cultures into the colon and withdrawing samples at various intervals and subjecting them to the procedure described. Thus, it was learned that *Bact. necrophorum* survived in a viable form for a length of time exceeding 12 hours, but less than 24 hours. (Table I.)

TABLE I.

Rate of Disappearance of *Bact. necrophorum* Experimentally Introduced into the Isolated Colon of Dog.

No. of Viable Organisms in Colon Specimen (Millions per cc.).

Exp.	No. Viable Organisms per cc. of inoculum	½	1	2	4	8	12	16	24 hr.
1	1	1	1	1	.1	.1	*	*	0
2	1	1	1	1	1	.1	.1	.01	0
3	1	1	.1	.1	.1	.1	.01	.01	0
4	10	.1	.1	.1	.1	.01	.01	*	0
5	1	1	1	1	.1	.1	.1	*	0

*Not determined.

The influence of oxygen upon *Bact. necrophorum* in the colon was studied; 50 cc. bacterial suspension were put into the loop and 5 minutes later oxygen was bubbled into it. The oxygen was obtained from a cylinder, the amount regulated by a valve, and was led into the colon through a sterile catheter after first being bubbled through water, in order to measure the volume of gas admitted. It was established that 18 bubbles expelled 1 cc. of water from a graduated cylinder. About 120 bubbles were injected per minute, over a period of 90 minutes, making a total of about 600 cc., some of which escaped through the fistula. Samples of loop-contents were then withdrawn and tested.

Bact. necrophorum were no longer demonstrable in the colonic segment after 90 minutes' treatment with oxygen.

To prove that the disappearance of the organisms was due to the oxygen and not to any mechanical action, such as distension of the colon and increased secretion and peristalsis, nitrogen was substituted for oxygen under identical conditions. The inert gas, nitrogen, did not affect the viability of *Bact. necrophorum.*

Cultures of *Bact. necrophorum,* in Rosenow's medium, were tested *in vitro.* Oxygen was bubbled through the medium at the rate of 120 bubbles per minute, and then samples were tested at intervals as before. It was found that oxygen for 90 minutes caused a great diminution in the number of these organisms, although 6 hours of this treatment was necessary to kill all of them.

Summary. *Bacterium necrophorum* introduced in large numbers into the isolated colon of a dog were recovered in appreciable num-

bers after a period of 12 hours. When oxygen was admitted into the bowel containing the culture for a period of 90 minutes no *Bact. necrophorum* organisms were recovered at the end of that time. Nitrogen similarly injected into the bowel was without effect.

9017 C

Absence of Vitamin E in the Royal Jelly of Bees.*

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In 1925, one of the writers (K.E.M.) working under the direction of the late Drs. T. B. Osborne and L. B. Mendel at the Connecticut Agricultural Experiment Station, attempted to assay the vitamin E-content of the royal jelly of the honey bee, which is the substance necessary for transformation of worker larvae into the queen or sexually productive form. Due to the relatively small amount of material procurable at the time, and to the negative data obtained, the results of these studies were not published. Since that time, Hill and Burdett[1] in England, claim to have demonstrated the presence of appreciable amounts of vitamin E in this interesting substance. However, there are many obvious objections to the method of assay used, and to the interpretations of results obtained by the latter investigators. They assume in the first place that, in normal stock females placed upon an E-deficient diet at the time of parturition, the process of suckling the litter would completely remove the stores of vitamin E in the maternal tissues. The fallacy of such an assumption is clear to all those who have had experience in the experimental production of vitamin E-deficiency. Furthermore, they state that out of 3 rats receiving a daily supplement of 50 mg. of royal jelly over a period of 37 days, 2 females were able to deliver litters of fully developed young. Out of 4 other rats, 3 of which received 2 gm. supplements of honey and pollen and 1 of which received 2 gm. of worker larvae brood comb, daily, only 1

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1 Hill, L., and Burdett, E. F., *Nature*, 1932, **130**, 540.