STUDIES ON PATHOGENIC B. COLI FROM BOVINE SOURCES.

I. The Pathogenic Action of Culture Filtrates.

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The strains used in these studies were all isolated from the ileum of young calves passing liquid feces and showing signs of a choleriform disease (scours). Most of the strains were obtained from sick calves, some moribund, immediately after they had been killed, a few after natural death. Several strains from normal calves were introduced for comparative study. They differ from one another in motility, fermentative capacities, the possession of capsules, tendency to mutate, and the like. More detailed descriptions will be given in papers to follow.

B. coli, representing a group, may be regarded as a special type of parasite, restricted to the digestive tube with occasional sallies into those organs or cavities where the protective forces of the host cannot be fully exerted, as in the urinary tract. It was thought that any new light which can be shed on its behavior might be useful in interpreting the behavior of the more highly specialized and parasitic paratyphoid group.

In various articles one of us has formulated the hypothesis that in the gradual evolution of pathogenic or invasive types of bacteria, the beginnings of parasitism may have been made possible by a soluble, diffusible toxin, but that in later stages this primary offensive, more or less accidental, mechanism is either partly or wholly suppressed and some different mechanism developed with which the bacteria protect themselves against the body-foreign forces of the host. The process may be regarded as shifting from the destructive, predatory to the parasitic, from the offensive to the defensive type. According to

this hypothesis, *B. coli* represents the early predatory, toxic stage, with, however, a certain specialization towards protection from anti-foreign activities in the digestive tract. It resembles in many respects the cholera vibrio in its activities. In view of the hypothesis presented, a study of any early appearing toxin in cultures seemed the first problem to be attacked.

EXPERIMENTAL.

Effect of a Living Culture on Calves.—In the following pages, the effect of culture filtrates on calves is the chief topic. As a preliminary, the notes on the effect of a living bouillon culture are here introduced.

A calf (No. 307), 32 days old, weighing 104 lbs., received into a jugular vein 2 cc. of a 24 hour bouillon culture of *B. coli* 223. This strain produced a large amount of viscid material, even within 24 hours. In 2 days, the entire bouillon became viscid, so that short, cobweb-like threads could be raised from the fluid. The calf was seen 25 minutes later. When seen again, 65 minutes after the injection, it was lying on its side, dead, and a mass of white froth about 12 inches long extended from its nostrils on the floor of the stall. The autopsy showed the following conditions.

Digestive tract pale throughout. Upper respiratory tract, including pharynx and larynx, cyanotic. Tracheal mucosa injected and covered with froth. Lungs large and heavy. Both large caudal lobes are intensely and uniformly congested, the condition bordering on hemorrhage. The condition as to blood content varies from lobule to lobule. The smaller lobes (ventral, cephalic) are far less congested and edematous. Subendocardial tissue, left side, around papillary muscles, infiltrated with blood in form of large patches. Liver with borders rounded. Over large areas there are confluent patches of a dark red color. These, on section, correspond to diffuse hemorrhage into parenchyma similar to the pulmonary lesions. Both kidneys have large dark red areas in the cortex. This hemorrhagic condition dips down in a linear way to medulla. Besides these radiating lines of hemorrhage, hemorrhagic patches are present, as in liver and lungs. Urine from bladder clear, amber-colored, and free from any visible blood tint. It contains a small amount of coagulable protein. In sections of lung, kidney, and liver the described lesions are shown to be due to an intense congestion or filling up of the capillary system associated with and merging into hemorrhage. When 16 days old this calf was fed with a heavy suspension of the same culture without showing disturbance of any kind.

Effect of Filtrates.—In a study of filtrates it was deemed best to restrict the experiments on calves to relatively young cultures. Veal broth containing 1 per cent peptone and 0.1 per cent dextrose was

sterilized in flasks in layers 2 to 3 cm. deep. 48 hours after inoculation the cultures were filtered through Berkefeld filters and the filtrate stored in full bottles at 38–40°F. until used. Any deterioration within several months was not observed.

The effect on calves 1 to 2 months old, on calves 6 to 7 months old, and on cows was qualitatively the same when the filtrate was injected into a jugular vein. Subcutaneous injection was without visible effect. After the intravenous injection of 2 cc. of filtrate the first signs in calves were manifest in from 5 to 20 minutes by a slight cough. After this there was a speedy increase in the number of respirations and pulse beats, the former rising to 100, rarely 120 or higher, the latter to 80 or even 100 and above. After 1 or more hours, both declined and the respirations became somewhat jerky. The expirations were usually interrupted and accentuated by a grunt. The temperature rose 1° or 2°C. after the injection but was normal the next day. The respiratory difficulties may in some animals become very great. The mouth is then held open, and saliva dripping from it, the head and neck held horizontal. Usually the calf is very restless, lies down and gets up repeatedly, or when very weak it lies on its side with the legs extended.

The reaction following the intravenous injection is, as might be supposed, not the same in all calves. A few failed to manifest the acute respiratory distress and the reaction showed itself in muscular tremors and chills. In most calves there were repeated discharges of semi-liquid feces in addition to the respiratory symptoms. The pronounced symptoms usually last 4 to 6 hours. Rarely the depression continues over 1 or 2 days. The following protocol is inserted to illustrate the time intervals of the several stages of the toxic effects.

10.20 a.m. Calf, 34 days old, Holstein female, receives, intravenously, 2 cc. filtrate of a 48 hour culture of *B. coli* 1085.

10.25 a.m. Calf very sick, lies down; respirations 130. Coughs frequently.

10.50 a.m. Respirations 88; pulse 60. Respiratory conditions the same.

11 a.m. Respirations 40. Temperature 38.9°C. Pulse 70.

12.15 p.m. Lying with head extended and legs straightened at right angles to body. An expiratory grunt as of some obstruction to expulsion of air.

3.15 p.m. Still lying in the same position. Temperature 39.1°C. A grunt with each expiration but not with any check in the movement. Respirations 70.

4 p.m. Temperature 39.4°C. Still lying down exhausted. Respiration as before. Copious discharge of feces.

5 p.m. Calf standing up. Brighter. Takes its evening food. Temperature 38.9°C.

Calf slightly depressed on the following day. Temperature about normal.

In calves 6 to 7 months old the same dose of 2 cc. produced severe reactions. One cow treated with the filtrates intravenously reacted severely after each injection of a dose increasing gradually to 15 cc. The filtrates of five strains of *B. coli* from calf scours distinguishable culturally from one another all produced the same succession of symptoms.

The mode of introduction of the filtrate naturally brings the respiratory tissues first under the influence of the toxin. The symptoms indicate an injury of the alveolar epithelium and vascular endothelium leading to increased permeability and transudation of fluid into the alveoli. That the toxin is a capillary poison is furthermore indicated by the lesions found in the fatal case following the injection of a living culture described above, and the following cases in which the autopsy showed the end effect on the lung tissue.

Holstein heifer calf, 48 days old, received into a jugular vein, a mixture of 2 cc. *B. coli* filtrate (1192b) and 6 cc. serum from a cow which had been treated with filtrate. This calf had received intravenously 2 cc. filtrate and 2 cc. serum when 38 days old. It went through the typical reaction associated with rapid respiration, open mouth and dribbling of saliva, 35 minutes after the injection. Dyspnea became pronounced and associated with a grunt during each expiration. The labored breathing continued from 11 a.m. until well into the night. The calf appeared free from any respiratory difficulties next morning. It was killed 2 days later. The viscera were normal with exception of the lungs which displayed an irregularly distributed congestion and small hemorrhagic areas. Sections showed the presence of deformed, cup-shaped red cells in small numbers in the alveoli of various regions. Distinct hemorrhagic areas were also present. In some lobules the alveoli contained granular and fibrillated material evidently fibrin. Polymorphs were loosely distributed in small numbers in the alveoli partly enmeshed in the alveolar coagulum. Mitoses of alveolar epithelium were not infrequent. This animal thus was still under the influence of a pulmonary congestion associated with small hemorrhages. There was no evidence of a persisting early or fetal pneumonia.

A Guernsey male calf, 4 weeks old, received into a jugular vein 2 cc. of a filtrate of *B. coli* 223, the same strain which had been fatal to Calf 307, 7 years ago. The filtrate was prepared from a bouillon culture 48 hours old.

The symptoms referable to the respiratory tract followed in the order described but with unusual intensity. The respiratory dyspnea came on within 30 minutes.

The calf breathed with mouth open. The respirations rose to 112, but dropped to 48 after 4 hours. At this time the calf was too weak to stand. The respiratory difficulty increased and the calf died about 5½ hours after the injection. The autopsy immediately after death showed besides a general congestion of the viscera, an intense congestion of the lungs. The right cephalic lobe was completely airless, heavy. On section the parenchyma was evidently filled with blood. The left cephalic lobe contained a little air. The interlobular tissue was broadened by hemorrhagic infiltration. The azygos lobe was in part airless through hemorrhages. Both large caudal lobes showed all degrees of congestion, edema, and hemorrhage from the still air-containing caudal tips to the cephalic margins of these lobes.

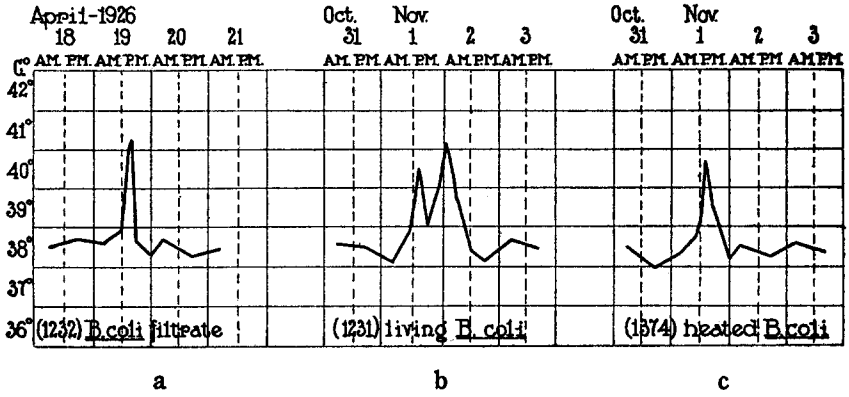
The spleen was enlarged through congestion. The kidneys were moderately congested, with a more deeply injected zone between cortex and medulla. Urine dribbling from the urethra during the early hours after injection of the filtrate was clear, slightly alkaline, specific gravity 1.002, with no protein coagulable by heat. Urine taken from the bladder soon after death had a specific gravity of 1.015, and contained coagulable protein equivalent to a deposit of 1.3 cc. in 10 cc. fluid.

Another calf received subcutaneously, at birth, 40 cc. serum from a cow treated with *B. coli* filtrates. When 29 days old and weighing about 112 pounds, it received into a jugular vein 2 cc. filtrate of a 48 hour bouillon culture of *B. coli* 1192a diluted with 3 cc. bouillon. The filtrate was 49 days old and it had been stored continuously at 36–40°F. in full bottles. Symptoms began within 20 minutes and ran the usual course with panting respirations rising to 120 per minute. The calf stood with head low, mouth open, and tongue protruding. 1½ hours after injection the respirations had fallen to 72. The animal was then very sick and unable to stand. Grunts with every expiration. 3½ hours after injection the animal began to be easier. Respirations 60, with occasional grunts. In 6 hours the reaction was nearly over and 1 hour later the evening meal of milk was taken readily.

The calf was killed about 23 hours after injection when in outwardly normal condition. The vessels of neck were severed after stunning the animal. The only organs visibly changed were the lungs. These were extensively involved. Three forms of lesions could be observed. (*a*) A dark red hepatization due to exudation into alveoli of blood and coagulation there. About 8 cm. of the free tip of the left cephalic lobe was in this condition. (*b*) Localized congestions and hemorrhages involving one or several lobules. These were scattered through the main (caudal) lobes chiefly. (*c*) Petechial hemorrhages 1 to 3 cm. apart, chiefly in the small cephalic lobes. The liver showed a patchy congestion visible both on surface and on section. Each liver cell contained one or more fat globules 2 to 5μ in diameter. Kidneys slightly congested and distinctly more moist on section than normal. Hyperemia of boundary zone between cortex and medulla. Spleen congested but normal markings still visible. Slight patchy hyperemia of upper small intestine. Urine about 30 minutes after injection contained about

1.3 per cent deposit of coagulable protein after heating. At autopsy there was only about 0.3 per cent. Urine 12 days before injection was normal.

Sections of fixed and hardened tissue presented nothing noteworthy beyond what is stated in the autopsy notes concerning the liver, spleen, and kidneys. In sections of the lungs, that portion found consolidated at autopsy presented various lesions. Focal hemorrhages were numerous and filled a group of contiguous alveoli with red cells. Forming a background for these hemorrhagic areas, the remainder of the alveoli contained a delicate network of fibrin fibrils holding enmeshed polymorphs in small numbers. The latter cells were brought together in denser groups within alveoli here and there. The other lobes of the lungs showed areas with partly collapsed alveoli and broadened walls as well as occasional small hemorrhages and alveolar fibrin. Polymorphs were thinly distributed throughout, both in the capillary bed and the alveolar lumina.



TEXT-FIG. 1. Temperature reactions in calves following the intravenous injection of *B. coli* and filtrates.

Thus far only filtrates of 48 hour bouillon cultures had been used. The presence of abundant toxin in a 24 hour culture was demonstrated on a calf 26 days old. After the intravenous injection of 2 cc. of the filtrate the sequence of symptoms already described appeared and with an intensity fully equal to that following the 48 hour culture filtrate. The calf was killed within 48 hours of the injection. The lungs still presented signs of the reaction in the form of subpleural hemorrhagic points and scattering congested lobules.

The effect of the intravenous injections on the rectal temperature of calves is shown in Text-fig. 1. The effect of a filtrate of a 48 hour bouillon culture is shown in (*a*). A similar unimodal curve (*c*) is pro-

duced by cultures heated at 60°C. for 30 minutes and therefore sterilized. In (*b*) the bimodal curve following the injection of living cultures may be due to immediate effects of the toxin followed by a temporary multiplication of the injected bacteria and hence a second dose of toxins.

The experiments reported were made with 24 and 48 hour culture filtrates. One test was made with a filtrate of a culture of 1192a incubated 8 days. The layer of bouillon in the flask was about 1 cm. deep. At the end of the incubation, the fluid was quite viscid and hence diluted with an equal volume of normal saline to facilitate passage through a Berkefeld filter.

4 cc. of the filtrate, containing 2 cc. of the original culture fluid, was injected into a jugular vein of a calf 34 days old. The symptoms followed one another as in the preceding cases but with much greater intensity and rapidity. The calf died in 3½ hours after the injection. There was complete hemorrhagic filling up of the entire left lung excepting a narrow margin of ventral and cephalic lobe. On section, the tissue was uniformly dark red, with reddish frothy fluid trickling off. The tissue was heavy but still resilient. The right lung, along median and dorsal region, was in the same condition; the lateral two-thirds of this lung was still partly air-containing with dark red areas in each lobule. Much foamy reddish fluid flowed from cut section of the pinkish regions. The trachea was filled with a reddish froth. There was a moderate congestion of the kidneys and some fat in the liver cells. The mucosa of intestines was only feebly reddened. The spleen was congested and weighed 460 gm.

This preliminary test clearly indicated a rise in the toxicity of the culture fluid due to longer incubation. Whether there is but one toxin involved or others superadded during the longer incubation remains unanswered for the present.

The effect of filtrates on guinea pigs introduced into the peritoneal cavity is relatively slight when compared with the serious effect on calves weighing about 100 pounds. The guinea pig weighing 350 to 600 gm. receiving the calf dose of 2 cc. into the peritoneal cavity reacts only with loss in weight as follows: Within 2 days there is a loss of 35 to 50 gm. in weight. Then there is a recovery so that in 7 days the original weight has not only been regained but added to by 10 to 15 gm. A small portion of this loss may be produced with bouillon alone. The injection of smaller doses, up to 0.5 cc., directly into the

heart failed to produce any acute symptoms or later death. In relation to body weight 0.5 cc. into the circulation of guinea pigs is over 30 times, the intraperitoneal dose of 2 cc. over 120 times the calf dose. In an early, fairly comprehensive study of calf scours, E. Joest[1] finding that filtrates of 24 hour bouillon cultures of *B. coli* were non-toxic for guinea pigs after intraperitoneal injection up to 3 cc., concludes that soluble poisonous products are not secreted.

It has already been stated that the subcutaneous injection of filtrates is without appreciable effect. The same is true when living cultures are introduced with the food. Two calves, about 2 months old, were fed *B. coli* in milk without showing any digestive or other disturbances. 500 cc. milk had been heavily seeded with a bouillon culture, warmed, and incubated for 7 hours. Plate cultures indicated ½ billion bacteria per cc. To disguise the flavor, fresh milk was added. The incubated milk coagulated when heated and was strongly acid to litmus.

Owing to obvious difficulties in multiplying experiments on calves only a few have been made bearing other phases. When bouillon cultures were shaken with kaolin and filtered there was no reduction of toxicity. When the filtrate was exposed to 80°C. for 30 minutes the toxicity though decidedly reduced was not completely destroyed.

One calf was treated with a 48 hour culture filtrate of a paratyphoid bacillus from guinea pigs.[2] 4 cc. in place of the usual 2 cc. were injected into a jugular vein. The symptoms following were similar to those produced by the *B. coli* filtrate, but less severe. The calf began to cough in 13 minutes. In 15 minutes the respirations were 104. In 30 minutes the calf was lying down, with respirations at 80 and evidently labored. After 1½ hours, respirations were 60 and dyspnea pronounced. Viscid saliva was hanging from the mouth. After 3 hours, the calf was lying quiet and apparently without distress. It took its milk after 6 hours. Next day it was still subdued. During the attack the temperature rose 1–1.5°C.

The soluble toxins of the large group of typhoid, paratyphoid, and colon bacilli have interested many observers since these groups were definitely recognized. Much of the work has been done in the paratyphoid group owing to its close relation to outbreaks of meat and

[1] Joest, E., *Z. Tiermed.*, 1903, vii, 377.

[2] Nelson, J. B., and Smith, T., *J. Exp. Med.*, 1927, xlv, 353; and Smith, T., and Nelson, J. B., *J. Exp. Med.*, 1927, xlv, 365.

other food poisoning. A fairly complete bibliography has been published recently by Miss Branham.[3] Among the recent papers which bear directly on the subject of this communication is one by Steinberg and Ecker[4] who prepared an antiserum in rabbits towards the soluble toxin with a culture fluid centrifuged but not filtered. The antiserum was tested upon rabbits inoculated with living cultures. The results apparently demonstrated the neutralizing power of the antiserum on the soluble toxin. An analysis of the experiments does not bear out the inference drawn. The rabbits received some living bacteria as antigen and the effect of the antiserum in the test rabbits may have been a suppression of the bacteria injected rather than a neutralization of the soluble toxin.

SUMMARY AND CONCLUSIONS.

The relatively young bouillon filtrates, 24 and 48 hours old, of certain strains of *B. coli* obtained directly from the ileum of scouring calves, were highly toxic for calves about 1 month old, as well as for older calves and cows when given into a vein. The symptoms, of panting followed by dyspneic and jerky respiration, indicate some at first obstructive action upon the alveolar and endothelial cells, followed by a greater permeability and eventual filling up of the air spaces with a serous, fibrinous, and hemorrhagic exudate. Similar effects are produced in other organs, such as liver and kidneys, if the toxin reaches them or is formed there by multiplying bacteria. There are no immediate or remote effects resembling those on calves following the intraperitoneal or the intracardiac injection of *B. coli* filtrates into guinea pigs even when the dose represents many multiples, per body weight, of the dangerous or even fatal calf dose.

The administration of the filtrate subcutaneously is without visible effect. Similarly, feeding large numbers of living bacilli produced no manifest disturbances.

In support of the hypothesis of a genetic relation between the group of *B. coli* and of paratyphoid, a similar but less severe effect was produced in a calf by the intravenous injection of a bouillon filtrate of a paratyphoid strain.

[3] Branham, S. E., *J. Infect. Dis.*, 1925, xxxvii, 291.

[4] Steinberg, B., and Ecker, E. E., *J. Exp. Med.*, 1926, xliii, 443.