STUDIES ON THE EFFECT OF CERTAIN TOXIC SUBSTANCES IN BACTERIAL CULTURES ON THE MOVEMENT OF THE INTESTINES.

I. The Effect of Soluble Toxic Substances of Young Cultures of Bacillus paratyphosus B.*

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Plate 28.

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HISTORICAL.

Since the classical studies of Gärtner (1) considerable work has been done to demonstrate the occurrence of toxins in cultures of organisms belonging to the paratyphoid-enteritidis group. The literature on this subject has been reviewed by Ecker (2) and by Branham (3). It has been shown by Ecker (2) and others that rabbits, upon intravenous injection of 2 to 5 cc. of a Berkefeld filtrate of young cultures of these organisms became weak, prostrated, and dyspneic in from 1 to 2 hours. The animals often exhibited a profuse diarrhea, while control animals receiving broth alone showed no symptoms. Smith and TenBroeck (4) in a study of the fowl typhoid bacillus observed that a dog receiving 1 cc. per kilo of a filtrate of a culture of their organism showed a diarrhea, with the passage of blood and mucus, and persistent vomiting. At the end of 24 hours there was a prolapse of the intestine and the animal was killed. On necropsy it was found that at least 2 feet of the small intestine had been invaginated through the ileocecal valve and had moved down through the large intestine and out through the anus. The authors believe that peristalsis is markedly stimulated by the filtrate. Geiger, Davis, and Benson (5) fed rabbits by stomach tube with food contaminated with organisms of the paratyphoid-enteritidis group and its filtrates, boiled and unboiled. Thirty rabbits were used for each experiment of each strain of organism. They observed symptoms of diarrhea once in a series of experiments with *B. paratyphosus* C, twice in the *B. paratyphosus* B series, and eight times in the *B. paratyphosus* A series. Of animals receiving fresh filtrates, diarrhea was noted

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in only two instances and these in the *B. paratyphosus* A series. We have not observed diarrhea in rabbits following feeding by stomach tube of 25 cc. of toxic filtrate of *B. paratyphosus* B, or *B. suipestifer*. Once a slight diarrhea was noted in a cat fed 25 cc. of a filtrate of *B. suipestifer*. We agree with Geiger, Davis, and Benson that rabbits have considerable tolerance to bacteria of the paratyphoid-enteritidis group and their filtered products, when placed directly into the gastrointestinal canal. However, upon intravenous injection we found that with certain strains of *B. paratyphosus* A and B, *B. suipestifer*, *B. enteritidis*, and *B. paratyphosus* C, diarrhea often occurs.

In the present study an attempt was made to observe directly the action of the toxic filtrates on intestinal movements because the existing data appeared unsatisfactory.

Friedberger and Kumagai (6) attempted to study the endotoxins of *B. typhosus*, *B. dysenteriæ* (Shiga), and the vibrio comma, on the isolated intestine by the method of Magnus, but failed to obtain a reaction. The intestines of both normal and immune animals were used. The only reaction they observed was when an anaphylatoxin was prepared. These authors claim that the homologous serum employed in the preparation of the anaphylatoxin did not affect the isolated segment.

It has been pointed out by de Graaff (7) that *B. paratyphosus* may produce para-oxyphenylethylamine and β indoleethylamine. Both of these compounds are known to be physiologically active. According to a recent study of Nakamura (8) para-oxyphenylethylamine in small doses tends to decrease the tone and movements of rabbit's intestines, while larger doses, following a distinct drop, produce a sudden rise of tone. Laidlaw (9) states that indoleethylamine has a direct stimulant action on plain muscle. Its physiologic action is intermediate between that of the sympathomimetic monamines, such as *p*-hydroxy-phenylethylamine, and the diamines, like imidazoleethylamine. With the Magnus method, Ecker and Siaulis[1] failed to obtain an effect on the isolated segment, indicating that we must be dealing with different substances. Furthermore, Branham reports that filtrates of *B. enteritidis* were found to contain a maximum of 0.000625 gm. of histamine in 200 cc. and that the usual amount injected was too small to have any but the most transient effect on the rabbit. Upon inoculation of material containing this trace of histamine, enough to equal the 2 cc. of the filtrate which they gave no effects were produced. It must be pointed out that the peptone may have contained this trace of histamine as no study of the broth alone was made by the author. Blankenhorn, Harmon, and Hanzlik (10) in a study of an outbreak of ptomain poisoning from "creamed codfish" reported that extracts of the "creamed" fish gave practically the same physiologic reactions as the same brand

[1] To be published.

of salted codfish which was previously allowed to putrefy and then was prepared in the same manner as the food served to the patients. The physiologic effects consisted of a marked stimulation of intestinal and uterine peristalsis with isolated surviving organs, and a fall of blood pressure with gradual recovery. The purified active extract of the creamed putrefied salted codfish contained some physiologically active base, whose chemical reactions resemble those of the group of diamines. The only organism found was *B. coli communior*. In these experiments the food was allowed to decay and it is therefore likely that active amino bases may occur, accounting for their results.

In our experiments young culture filtrates (less than 24 hours) have been employed and all the experiments have been carried out with the organ *in situ*, in the living animal.

Method.

Several strains of *B. paratyphosus* B were employed. Nos. 180 and 185 gave the most satisfactory toxins and No. 180 was particularly active in the production of a diarrhea. No. 185 caused a more violent respiratory reaction. The organisms were grown for 15 to 24 hours at 37°C., on 2 per cent Witte peptone veal infusion broth with a pH of ± 7. Witte peptone gave the most satisfactory results. The cultures were then filtered through a Berkefeld N candle and were always used immediately after filtration. In the early experiments we have grown Nos. 180 and 185 together and have also mixed equal volumes of the two filtrates to obtain maximal effects, but the filtrate of either strain will cause the same marked reactions, considering variability of toxicity of the filtrate and susceptibility of the animal. The sterile broth was used as a control. Paralleling these experiments normal rabbits (2 to 3 kilos) were given intravenous injections of the filtrates in order to ascertain their activity. The animals destined for operation were anesthetized and rendered immobile by a large dose of urethane (2 gm. per kilo) administered by stomach tube. The animals were given no food for at least 24 hours prior to the experiment.

The first method used for the study of the movements of the small intestines *in situ* in the living animal was the "pouch method" of Sollmann (11). An incision, about 15 cm. in length, was made through the skin of the abdomen in the median line. Following blunt dissection of the skin from its underlying fascia, the skin was suspended from an oval ring measuring 15 by 17 cm. The ring was then raised with a suitable V-shaped support to form a water-tight pouch. The sac was filled with warm Locke's solution,[2] carefully kept at constant temperature

[2] The solution used by us contains, per liter H_2O: NaCl 9.0 gm.; KCl 0.42 gm.; $CaCl_2 2H_2O$, 0.24 gm.; $NaHCO_3$ 0.3 gm.; glucose 1 gm. pH = 7.8.

(38.5–39°C.) by inflowing warm Locke's solution. The excess fluid flowed over the border of the ring. An incision (± 3 cm.) was then made through the wall of the abdomen and a loop of the small intestine with a fairly large mesentery was brought out. The wound was sutured to prevent the escape of other loops. The selected segment was then horizontally hooked up between two fixed pins and by means of a serrefine or pin, thread and lever the pendulum movements were recorded in the usual way. The lever registrations were always supplemented by direct inspection. Following preparation of the animal, from ½ to 1 hour was allowed to elapse before any registration was made and the experiments were begun when the pendulum movements were regular and free from spontaneous changes. At times 2 hours were necessary to await the disappearance of spontaneous changes and an occasional animal was encountered in which the irregularities of contraction were such as to make experimentation impossible.

In the study of the propulsive efficiency of the normal segments *in situ*, and in the living animal, we employed the Sollmann and Rademaekers (12) modification of an arrangement described by Max Baur (13). The technique with this arrangement is more complicated and for its description the reader is referred to the original publication. By this method we were able simultaneously to study and record not only the longitudinal and circular muscle contractions but also the propulsive efficiency of the segment for a given time.

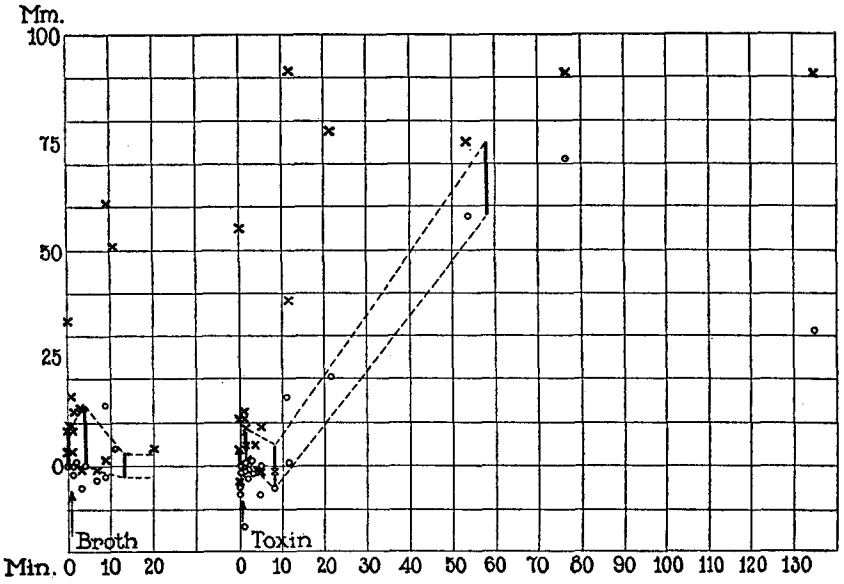
In charting the results of these experiments we have measured in mm. the "diastolic" and "systolic" tones at points of definite changes. These were plotted on the ordinates and the time in minutes on the abscissæ. The median was then taken to indicate the changes.

The Effect of Intravenous Injection of Filtrates on the Pendulum Movements of the Upper Intestine.

In a series of five experiments with parallel controls the rabbits were first injected intravenously with sterile broth (2 to 5 cc.) to study the effect of the broth. A slight rise of tone occurred immediately after intravenous injection of the broth. A similar reaction was seen when physiologic salt solution was injected or a simple puncture of the vein was made. Shaving of the ear may induce identical results. This effect never lasted more than 3 or 4 minutes following the injection of the broth, when the contractions returned to normal or slightly below. Text-fig. 1 shows that a systolic rise of 6 mm. occurred in these animals (median of five experiments) which at the end of 4 minutes disappeared again. We have always allowed from 1 to 3 hours to elapse after the injection of the broth and before the toxic filtrate was administered in order to ascertain whether or not

the broth itself may produce a late rise in tone. This did not occur in any case, thus confirming the clinical observations. A dose of 10 cc. of sterile broth in the clinical experiments failed to produce any symptoms in the animals.

Text-fig. 2 shows the effect of the filtrate on the tone of the longitudinal muscles. It is noted that the same immediate rise of sys-



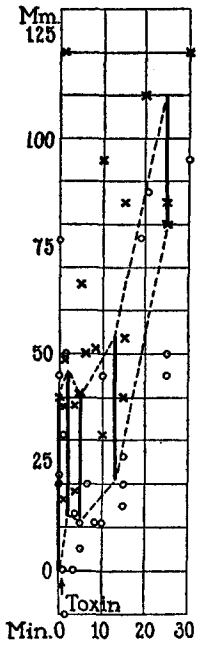
TEXT-FIG. 1. TEXT-FIG. 2.

TEXT-FIG. 1. The effect of intravenous injection of sterile broth on the tone of the longitudinal muscles of the upper intestines of the rabbit. (Median of five experiments.)

TEXT-FIG. 2. The effect of the toxic filtrate on the longitudinal muscles of the upper intestines of the rabbit. Preceding the marked rise of tone an immediate slight rise of the tone due to the injection is observed. (Median of five experiments.)

tolic tone occurred. Here the drop came at the end of the 2nd minute and progressed in a further drop to a diastolic of -5 and a systolic of $+5$, by the end of the 8th minute. From here on the phenomena changed to a second, slow but vastly powerful, rise of tone reaching its maximum at the end of 58 minutes. The diastolic

rise reached 58 mm. and the systolic 70 mm. The reaction was persistent in character as demonstrated in Text-fig. 4. In Text-fig. 2 the reaction was late in two of the animals. One of these (slower one) received a phenol-containing toxic filtrate. We do not know whether this accounts for its late effect or not.



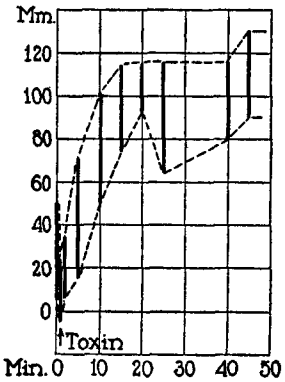
TEXT-FIG. 3. Showing the increase of tone of the longitudinal muscles of the upper intestines of the rabbit due to the toxic filtrate injection. No previous injection of broth was given. (Median of five experiments.)

In a subsequent series of five animals no preliminary broth injections were given. The animals received the usual amount of the toxic filtrates in the ear vein. Prior to the experiment, intact control animals received the same filtrates to determine their toxicity. The reaction in these animals was acute and diarrhea was always present. Text-fig. 3 illustrates the results obtained. Again the median of five experiments was plotted. The normal amplitude here was large and following the same immediate rise (broth effect) the tone gradually rose reaching its maximum at the end of 25 minutes. The amplitude at this point was shorter; *i.e.*, the spasm was such that the intestine relaxed but little between peristaltic waves. The diastolic rise was from 11 mm. to 80 mm. and the systolic from 42 mm. to 110 mm. A definite rise occurred at the end of 13 minutes (diastolic from 11 mm. to 21 mm. and systolic from 42 mm. to 54 mm.). An incubation period of a minimum of at least 10 minutes passed before the rise was noted. This period varies in different animals and with different filtrates.

Text-fig. 4 shows the persistency of the rise. Fluctuations of course occur but the tone remains high and in this case a still higher point was reached at the end of 50 minutes. The toxic filtrate employed in this experiment contained phenol (0.4 per cent), which accounts for the immediate brief drop of tone following injection of the filtrate.

We have further observed instances of a persistent high tone lasting for 2 hours, following intoxication.

It was found that magnesium chloride or sulfate, 5 to 10 cc. of isotonic solutions ($MgCl_2$ 2.1 per cent and $MgSO_4$ 3.25 per cent) added directly to the bath, relaxed the toxin spasm and quieted the excessive peristalsis. Text-fig. 5 (mean of four experiments) clearly demonstrates the effect of the salts. Within 14 minutes after the addition of the salt the tone dropped and with this drop the amplitude was also decreased. The diastolic tone of the intestine was 50 mm., the systolic 110 mm., and the amplitude therefore 60 mm. At the end of 14 minutes the diastolic tone was 13 mm., the systolic 22 mm., and the amplitude 9 mm.



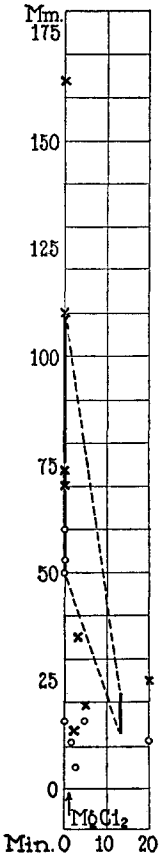
TEXT-FIG. 4. Showing the persistency of a high tone of the longitudinal muscles of the upper intestines of the rabbit following the administration of the toxic filtrate.

In Tracings 1, *A*, *B*, and *C* (longitudinal muscle movements) the original contractions were small. The effects of broth and toxin injection are clearly demonstrated. Tracing 1, *B* shows the rise of tone and Tracing 1, *C* shows the maximal effects obtained. Evidence of peristalsis is here seen. Peristalsis occurred during the rise.

The Effect of the Filtrate on Peristalsis and Propulsion in the Small Intestine.

The preceding experiments have definitely shown that the toxic filtrates stimulate the longitudinal muscle of rabbits' intestine. Since it is not known, however, that the longitudinal muscle always reflects

the activity of the circular muscles, on which peristalsis depends, another series of experiments was made in which the peristaltic propulsion was measured by the propulsion arrangement. With this method, an interval of usually 2 to 3 hours elapses before good peristalsis becomes established. Dr. Sollmann thinks that this is due to depression of the intestinal muscles by the large dose of urethane that is used to secure complete immobilization of the animal. This is gradually washed out by the peritoneal lavage, so that the intestines recover their activity. In the interval, the intestinal tone is low. Sollmann and Rademaekers observed that 6 to 8 cm. of water failed to provoke peristalsis under these circumstances. It is therefore of particular interest that the toxic filtrate injected during this period almost invariably started the peristaltic waves. Again, in these experiments the effect of sterile broth injections on the propulsive efficiency of the segment was carefully studied, and compared with the effect of a toxic filtrate injection. In other experiments we have allowed sufficient time to elapse following administration of the broth to overcome the temporary inhibition of peristalsis and when the waves became regular the filtrate was injected. Lastly, the toxic filtrate was injected without any previous broth inoculations. All these experiments were controlled with unoperated animals, which received the same amount of the filtrates.



TEXT-FIG. 5. Showing the relaxing effect of isotonic magnesium chloride. (Median of four experiments.)

In Experiment III (output arrangement), the rabbit, 1950 gm., received 4.5 gm. urethane by stomach tube, 3 cc. broth by ear vein, and later 3 cc. of an 18 hour toxic culture filtrate of *B. paratyphosus* B Nos. 180 and 185. The quantity of fluid propelled by the peristalsis of the intestinal segment was increased to about three and one-half times by the toxin. 84 dipping buckets were discharged in 108 minutes following the injection of the broth; but 298 buckets in the same time (109 minutes) following the injection of the filtrate.

Tracings A, B, and C of Fig. 2 demonstrate the reaction.

In a second experiment, No. IV, under the same conditions (rabbit 2600 gm. 5 gm. of urethane, 5 cc. of a 15½ hour culture filtrate of *B. paratyphosus* B Nos 180 and 185) the output was increased to four times, *i.e.* from 25 buckets in 100 minutes following broth administration to 126 buckets in 120 minutes, thus about 1 bucket per minute. In this case the waves occurred before the sterile broth was injected. Two control animals showed marked defecation from ½ to 1 hour following injection of the toxin, and at night soft stools were evacuated by these animals. They exhibited the same typical picture of intoxication.

In a third experiment of this kind (rabbit 1600 gm., urethane 3.0 gm., filtrate of 19 hour culture of *B. paratyphosus* B Nos. 180 and 185, 4 cc. intravenously), the output started 26 minutes after the injection of the toxic filtrate. No preliminary broth injection was given. In a total of 127 minutes the segment dumped 27 buckets. A control rabbit of the same weight evacuated soft feces 55 minutes after the injection of the toxic filtrate, and exhibited all the usual symptoms. 3 hours after the injection this control animal evacuated markedly. In Experiment VI with the same arrangement (rabbit 1350 gm., 3.0 gm. of urethane, filtrate of 18 hour culture of *B. paratyphosus* B Nos. 180 and 185), the animal received two injections, each time 3 cc. sterile broth, and sufficient time was allowed to elapse so as to obtain a normal peristalsis. The second broth injection was given 1 hour and 15 minutes after the first. Following the second injection the output was, in 56 minutes, 62 buckets, and following the toxin injection it was, in 88 minutes, 93 buckets, thus not so marked an increase as in the previous experiments. A control rabbit weighing 1870 gm. received the same amount of the toxic filtrate and showed a marked reaction with typical diarrhea in 1 hour after the injection of the toxic filtrate. The interesting part of this experiment is the fact that the waves were spastic, travelling slowly, following the injection of the toxic filtrate, and the output therefore not greatly increased.

A mild effect was obtained with the filtrate of a strain of an organism of the paratyphoid group freshly isolated from a food poisoning outbreak.

Incidentally, it was also observed that the rabbits under urethane did not exhibit as much diarrhea as the control animals without anesthetic. In one instance a most profuse diarrhea occurred in one animal after it came out of the anesthesia. In other words, the urethane tends to diminish the diarrhea.

SUMMARY.

Following intravenous injection, filtrates of young cultures of *B. paratyphosus* B often produce marked diarrhea in rabbits. A study was made of the effect of these toxic filtrates on the motility

of the small intestines of the rabbit. The observations were made on a segment of the small intestines *in situ*, and in the living animal. It was found that an immediate slight rise of tone of the longitudinal muscles occurred following intravenous injection of sterile broth. The same rise was noted after the injection of the toxic filtrate; but with these it was followed later (10 minutes elapsing at least) by a very strong but gradual rise of the diastolic and systolic tone, *i.e.*, by spasmodic contraction of the intestinal muscle, which persisted at times for as long as 2 hours. In order to record simultaneously the effect on the longitudinal and circular muscles, and the propulsive efficiency of the segment the Sollmann and Rademaekers modification of Baur's technique was employed. This arrangement showed that the stimulation of the longitudinal muscles is accompanied by a similarly strong stimulation of the circular muscles, by peristalsis, and therefore by a greatly increased propulsion of intestinal contents which was sufficient to overcome the inhibition that usually occurs after preparation of the animal. With this arrangement an instance of peristaltic spasm was also noted. Broth alone failed to produce the phenomenon. Isotonic magnesium chloride or sulfate added to the bath relaxed the muscles again. Animals under deep urethane anesthesia did not show the diarrhea occurring in the intact controls, but sometimes exhibited it after the effect of the anesthetic had disappeared. So far no effects have been observed on the isolated strip (Magnus method), and further studies are being made to localize the effect, to neutralize it with a specific antiserum, and to observe the effect of filtrates of other members of the bacterial group including the dysentery bacilli.

We desire to acknowledge our gratitude to Dr. T. Sollmann for his continuous interest in the progress of the work.

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EXPLANATION OF PLATE 28.

FIG. 1 (tracings). The effect of the toxic filtrates on the pendulum movements of the upper intestines of the rabbit. (*A*) Demonstrating the immediate rise of tone and amplitude obtained after the injection of the broth and filtrate. (*B*) Demonstrating a marked increase of tone and amplitude due to the injection of the toxic filtrate. (*C*) Demonstrating the maximal effect produced. Peristaltic waves were observed.

FIG. 2 (tracings). The effect of the toxic filtrates on intestinal movements. Pendulum, circular, and propulsion. (*A*) Demonstrating the effect of sterile broth. (*B*) The effect of the toxic filtrate. (*C*) Maximal reaction obtained. The output is almost continuous.

Uppermost tracing: longitudinal contractions.

Second tracing: pressure curve.

Third tracing: propulsion.

Fourth tracing: time in minutes.

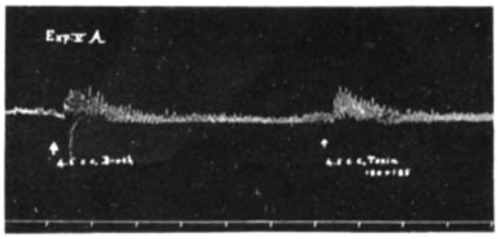

FIG. 1, *A*.

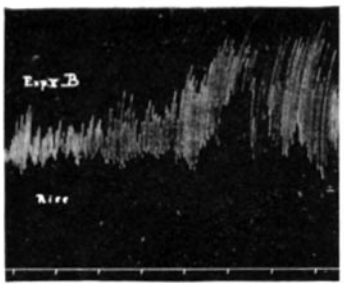

FIG. 1, *B*.

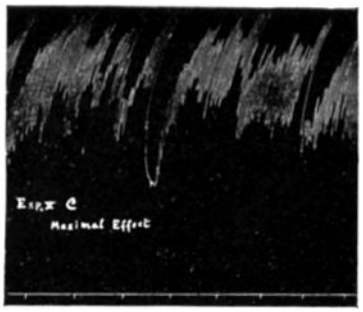

FIG. 1, *C*.

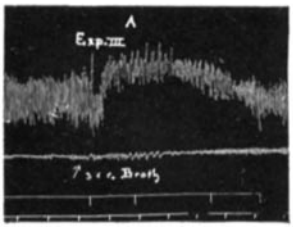

FIG. 2, *A*.

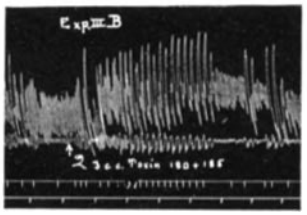

FIG. 2, *B*.

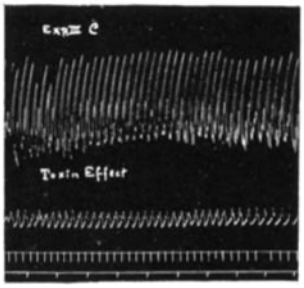

FIG. 2, *C*.

(Ecker and Rademaekers: Toxic substances in bacterial cultures. I.)