negative after-potentials fail to pass the zero level even at their maxima. The course of events is illustrated in Fig. 2. At the break in the line the tetanus starts. Only the after-potentials are visible, the width of the white band of the record being determined by the increments of negative after-potential. If swept out the component responses would look like Fig. 1 B. The top of the band is determined by the negative after-potential crest and the bottom of the band by the amount to which the potential has subsided at the time of arrival of the succeeding impulse. As the tetanus proceeds the positivity at first increases; then the 2 potentials come into balance and a steady state is maintained on the positive side of zero. When the tetanus ceases the negative after-potential subsides much earlier, revealing the positive potential unopposed. In the subsequent restoration of the potential to the resting value 2 parts are seen which are also characteristic of unpoisoned nerve[2]: a rapid portion manifest initially *(P1)* and a slower portion which completes the restoration *(P2)*.

For contrast with the potential accumulation in a yohimbinized nerve during a tetanus, the accumulation of potential as seen in a veratrinized nerve in a similar experiment is shown in Fig. 3. Negative after-potential is here dominant, and the level of the latter rises continuously during the tetanus and lasts long after the latter has ended. In fact, in some nerves the crest of the negative after-potential is not attained until after the tetanus is over.

## 7762 C

## A Fermentation Inhibiting Substance Produced by B. Coli.

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When diphtheria bacilli were grown in broth media in which colon bacilli had previously been cultivated, the fermentation of dextrose, galactose and dextrin was inhibited or retarded. Usually this retardation was evident over a period of about 7 days and at the end of this time fermentation began to become evident. This inhibitory effect of the previous growth of *B. coli* on the fermentative activity of *C. diphtheria* was only evident when small batches of media (150 to 175 cc.) were exposed to the action of *B. coli*. In larger batches of media this inhibitory effect did not develop.

Colon bacilli were inoculated into sterile solutions of peptone, beef extract or meat infusion (both before and after removing solids and coagulable material) plus 0.5% sodium chloride and then incubated 48 hours. The media were centrifuged to remove bacteria as far as possible; the ingredient lacking (either peptone or beef extract) was added, the reaction brought to pH 7.4-7.6 and bromcresol-purple was added as indicator. The media were then filtered through either Berkefeld or Seitz filters to render them sterile. The carbohydrates were added either before or after filtration in quantities sufficient to make a 1% concentration.

The inhibiting effect on fermentation by diphtheria bacilli was evident in all media prepared as above; the results of one experiment are shown in Table I. Six different strains of *B. coli* of the fecal

TABLE I.

| | Diphtheria Culture | Days of Incubation. Dextrose | | | | Galactose | | | | Dextrin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | 1 | 2 | 4 | 7 | 1 | 2 | 4 | 7 | 1 | 2 | 4 | 7 |
| *B. coli* | 1 | — | — | — | A— | — | — | — | — | — | — | — | — |
| filtrate | 3 | — | — | — | — | — | — | — | — | — | — | — | — |
| in nutrient | 12 | — | — | — | A— | — | — | — | — | — | — | — | — |
| broth | 19 | — | — | ± | A | — | — | — | — | — | — | ± | A |
| *B. coli* | 1 | — | — | — | A | — | — | — | ± | — | — | — | A |
| filtrate in | 3 | — | — | — | — | — | — | — | — | — | — | — | — |
| peptone | 12 | ± | A | A | A | — | — | ± | A | — | — | ± | A |
| water | 19 | — | — | — | A | — | — | — | ± | — | — | — | — |
| *B. coli* | 1 | — | — | — | A | — | — | — | — | — | — | ± | A |
| filtrate | 3 | — | — | ± | A | — | — | ± | A | — | — | — | — |
| in beef | 12 | — | — | ± | A | — | — | — | ± | — | — | A | A |
| extract | 19 | — | — | ± | A | — | — | A | A | — | — | — | A |
| Control | 1 | A | A | A | A | — | A | A | A | — | A— | A | A |
| plain | 3 | A | A | A | A | ± | A | A | A | ± | A | A | A |
| broth | 12 | A | A | A | A | A | A | A | A | A | A | A | A |
| | 19 | A | A | A | A | A | A | A | A | A | A | A | A |

— No acid produced. ± Slightly acid. A Acid.

type were used and all produced essentially the same inhibiting effect. Several strains of *C. diphtheriae* were tested and the influence of the filtrate was evident on the fermentation activity of all. There were slight variations in the production of inhibiting influence by the strains of colon bacilli and also in the resistance of the individual strains of diphtheria bacilli to the inhibition. These colon bacilli filtrates did not interfere with the multiplication of the diphtheria bacilli.

An experiment was performed to determine whether the *B. coli* had exhausted the peptone. Peptone in solid form and in sufficient

quantity to make a 0.5% solution was added to a broth filtrate or peptone water filtrate in which *B. coli* had been grown. In this media the inhibiting effect was also evident.

Since many of the proteolytic enzymes of *B. coli* are extracellular, the filtrate of a 30-hour culture of *B. coli* was added to sterile broth and peptone water and incubated for 3 days. The mixture became slightly alkaline, it was adjusted to pH 7.4-7.6; lacking ingredients were added as well as the indicator and the carbohydrates. When such media were inoculated with *C. diphtheriae,* fermentation of the three sugars proceeded as in the control tubes.

In order to determine whether the diphtheria bacilli were altered by growth in the *B. coli* filtrate, cultures of *C. diphtheriae* were made on blood agar plates from tubes in which fermentation had been inhibited; the colonies on these plates were identical with the original colony forms, and when these organisms were inoculated into control carbohydrate media the fermentation was as active as in the original culture. If, however, the diphtheria organisms were inoculated directly from filtrate media, in which inhibition had occurred, into control carbohydrate media, there was evidence of a slight retardation of the fermentative activity of *C. diphtheriae,* so that in some cases 48 to 72 hours were required for activity to appear; if a second transfer was now made to normal carbohydrate media, fermentation of the 3 carbohydrates occurred within 24 hours. If the *C. diphtheriae* were transferred into normal control carbohydrate media from the *B. coli* filtrate media after 7 days' incubation, little or no inhibiting effect was evident. It is probable that the inhibiting effect had been destroyed or neutralized at this time, since this corresponds to the time at which fermentation of the carbohydrates begins in the *B. coli* filtrate media.

Heating the *B. coli* filtrate showed that the inhibiting effect is not readily destroyed by heat. It is apparent that the variation in influence of the inhibiting action on different strains of diphtheria bacilli plays a rôle. In addition, heat apparently destroys most readily the inhibitory action in relation to dextrose, and least readily the action in relation to dextrin. Even when heated to 115-120°C. the inhibiting action of the *B. coli* filtrate may not be completely destroyed.

It was also found that when the filtrate was diluted with normal nutrient broth it was necessary to have present more than 50% of the filtrate in order to have the inhibiting effect evident; so that usually when the filtrate constituted 75% of the mixture the effect was evident.

*B. coli* was grown in synthetic media (Mavor's and Uschinsky's); the filtrates so obtained from the synthetic media were each mixed with one part of nutrient broth, to aid growth. In both of these mixtures the fermentation by *C. diphtheriae* was not interfered with or retarded.

The *B. coli* filtrate was placed in contact with kaolin at various hydrogen-ion concentrations, but the inhibiting substance was not removed.

*Summary.* *B. coli* produces, when grown in small batches of nutrient broth, a relatively thermostable substance which inhibits or retards fermentation of dextrose, galactose, and dextrin by *C. diphtheriae*. The diphtheria bacilli seem to absorb this substance and also to destroy it, and there is no permanent effect of this substance upon the diphtheria bacilli. The action is not dependent upon exhaustion of nutrient substances or alteration in the pH of the media.

7763 P

## Lead IV of the Electrocardiogram in Rheumatic Fever.

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The most important clinical problem which presents itself in patients suffering from rheumatic fever is the recognition of active cardiac involvement. Bedside observation is sometimes adequate for decisive diagnosis. Serial electrocardiography, with employment of the 3 standard leads, has proved to be a valuable method for following the effect of the rheumatic process on the heart.[1] We have found that the additional use of lead IV has, in certain instances, revealed evidence of active carditis when in the first 3 derivations either no form changes were apparent in successive records, or the alterations noted were regarded as equivocal.

Thirty-eight patients with acute rheumatic fever were studied in the wards of the Presbyterian Hospital. None of these patients were taking digitalis or quinidine, although to a majority salicylate or pyramidon was being given. Two hundred and ten electrocardiograms were taken. In recording lead IV, the right arm elec-

[1] Cohn, A. E., and Swift, H. F., *J. Exp. Med.*, 1924, **39**, 1.