STUDIES ON THE DEVELOPMENT OF TOXICITY IN INTESTINAL SECRETION.

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The following study was conducted with the purpose of further investigating the production of toxic material in closed loops of high intestine in dogs. These experiments are a continuation of the work of Davis,\(^1\) and of Stone, Bernheim, and Whipple.\(^2\) In the work of Davis, intestinal secretion, collected by irrigation of the duodeno-jejunum, excluding pancreatic secretion, was kept under chloroform and toluene a number of days in a warm place, and studied for its toxic properties. These fluids were found to be toxic, and it was felt that the intestinal secretion was, possibly, toxic at the moment of its elaboration. In the light of subsequent work, however, the author feels that his conclusions are subject to revision. The condition under which the fluid was kept did not absolutely exclude the possibility of bacterial growth. Whipple, Stone, and Bernheim\(^3\) studied the toxic properties of fluids collected in closed loops of intestine left in situ, and certain inferences were reached as to the source of the toxin in these fluids. The present article deals with a further effort to investigate these inferences, in consequence of which light has been thrown on them from a different point of view. In the work referred to, the rapidity with which toxins developed,


the generally assumed paucity of bacteria in high intestinal loops, 
and certain other reasons, led to the assumption that the activity 
of the mucous membrane of closed loops was the essential factor 
in toxin production, and that bacteria probably played a minor part, 
if any, in this process. Our present data justify the conclusion that 
toxins indistinguishable, as far as the symptoms and anatomical 
lesions produced by them are concerned, from those found in closed 
loops, may be elaborated from intestinal secretion in vitro, and hence 
make questionable the theory of the essential part played by the 
living mucosa of closed loops.

Toxicity of Intestinal Secretion Heated to 90–95°C.

As a point of departure for this work we desired to determine 
whether intestinal loop secretion, obtained in such a manner that it 
was as nearly normal as possible, was in itself toxic. In order to do 
this, it was necessary not only to make an effort to exclude bacterial 
action, as by the addition of chloroform and toluene, but also to elim-
ninate the action of enzymes present in the secretion. For this pur-
pose the following experiment was devised. Under anesthesia, the 
intestine was divided just distal to the lower pancreatic duct, and 
again at a point in the high jejunum so as to isolate a loop of duo-
denojejunum. The upper and lower ends of this loop were brought 
out of the abdominal cavity, and connected with rubber tubing so 
that the isolated loop could be washed through and the washings 
collected. The loops were irrigated with distilled water. A given 
volume of water, usually 1 liter, was passed through the loop repeat-
edly so that large quantities of fluid would not have to be handled. 
A number of experiments were carried out with this operative tech-
nique, the loop being washed by a slow, continuous stream for periods 
ranging from $3\frac{1}{2}$ to 10 hours. The washings were collected over a 
boiling water bath, the temperature in the collecting flask being 
90–95°C. This temperature may be assumed to be destructive of 
both enzymes and bacteria, so that fluid so collected and reheated 
for $\frac{1}{2}$ hour on the 2 succeeding days, to allow for the presence of spore-
bearing organisms, remains unchanged, if kept with aseptic precau-
tions. Fluid so collected was injected intravenously into small
dogs, in two experiments, and produced no symptoms whatever.
In other experiments, conducted under anesthesia, injection of this
fluid caused no change in blood pressure.

Toxicity of Unheated Intestinal Secretion.

It might be suspected, however, that the heating itself destroyed
a toxin normally present in intestinal secretion. To eliminate this
possibility, the washings were collected as described above, except
that they were not heated. This fluid was immediately injected
intravenously into a small dog, also without the production of any
symptoms. In addition, in two other experiments, similar unheated
fluids, which had been preserved for a time by the addition of chloro-
form and toluene, were injected after driving off these substances
at a comparatively low temperature with the aid of reduced pressure,
without producing symptoms. From these experiments, we concluded
that the intestinal secretion, as produced in these loops is devoid of
toxic properties, and that if it is heated immediately at 90-95°C. and
kept sterile, the development of toxic properties is prevented.

The next step was to discover whether this non-toxic fluid would
take on, outside the body, the toxic properties characteristic of closed
loop contents. Unheated loop washings, collected in sterile flasks
and kept as far as possible from external contamination, were incu-
bated at 37°C. for 18 hours. The fluids at this time showed evident
and profuse bacterial growth. Upon intravenous injection, in two
experiments, these incubated washings caused the death, in about
6 hours, of small dogs. In both cases the symptoms and anatomical
pictures were the same as those seen after the injection of closed loop
contents. The small intestine was intensely injected and hemor-
rhagic, most markedly in the upper duodenum. In one case, the
quantity used represented the collection during 40 minutes, and in
the other during 80 minutes.

Toxicity of Unheated Intestinal Secretion Treated with Chloroform and
Toluene.

The development of toxic properties by the loop washings might
be ascribed to the influence of bacterial growth or to the action of
enzymes, probably proteolytic, derived from the intestinal mucosa. In either case, the substrate would have to be the proteins of the suc-
cus entericus, no other substance except distilled water being present. A method of differentiation by which either enzymic or bacterial action alone could be made responsible for the change is difficult to devise. Passage of loop washings through a Berkefeld filter was not attempted, since the adsorption of a large part or all of the enzymes by the material of the filter might be expected.

Fractional sterilization of the loop washings offered little, since growth of bacteria occurs between sterilizations, and in addition the temperature of sterilization approaches that at which enzymes are injured.

Finally experiments were carried out in which unheated loop washings were treated with chloroform and toluene. Portions, in one case one-half the amount collected in 3½ hours, and in one case one-third the amount collected in 4 hours, were kept several days and then freed of the preservative substances by distillation. The second portion had in addition been incubated at 37°C. for 18 hours. It was hoped that this procedure, while preventing bacterial growth, would allow opportunity for any enzymes present to act. The fluids were injected intravenously immediately after the distillation, and no symptoms of poisoning were observed in the dogs used.

The other portions of loop washings from the experiments concerned were incubated for 18 hours at 37°C., after being freed from the preservatives by distillation at reduced pressure, and then injected. In one case in which the distillation was carried out at a temperature of about 80°C. the injection of the fluid in a test dog produced no symptoms of poisoning. In the other, a better vacuum was obtained, and the temperature of the distillation never exceeded 60°C. The injection of this fluid produced a period of shallow respirations and weak pulse during the injection, but at the end the animal was in good condition and ran about the floor before being put in the cage. Extreme prostration supervened in 4 hours and the dog died during the ensuing night. There was no diarrhea. At autopsy the intestine was pale and showed nothing abnormal except the presence of a rather large amount of gas. The content of the small intestine was a bile-stained fluid. The mucosa showed no
hemorrhage or congestion. This picture is entirely unlike that caused by the injection of closed loop fluid, and has never been observed by either of the authors in long series of such injections.

In both these last experiments, the fluids, after removal of the preservatives and incubation for 18 hours, showed growth of *Bacillus subtilis* in spite of the precautions taken. It is probable that the spores of this organism survived the treatment with chloroform and toluene.

From these experiments, we cannot say positively that bacteria are responsible for the development of toxicity in loop washings, but they offer no support to the conception that enzymes are the cause of such development. These experiments, together with the previous ones reported by one of us (Davis⁴), show that loop washings can become toxic outside the body, producing symptoms like those caused by closed loop fluid. In this case there can be no question of the perverted activity of the epithelial cells of the mucosa. In addition, it is clear that unless chloroform and toluene inhibit the enzymes in this intestinal fluid, their action alone does not produce the toxic properties.

**SUMMARY.**

1. Intestinal secretion, collected by the method described in this paper, is non-toxic when fresh.
2. This secretion, when heated immediately to 90–95°C. and kept sterile, remains non-toxic.
3. This secretion, when not heated, remains non-toxic when kept under chloroform and toluene, even if incubated at 37°C.
4. This secretion, when not heated, but collected in a sterile flask, becomes toxic upon incubating 18 hours, producing symptoms like those of closed loop fluid.
5. The secretion, when treated with chloroform and toluene, and later incubated for 18 hours, after these preservatives have been removed by distillation at 60°C., does not produce lesions typical of closed loop fluid.