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THE EFFECT OF INSULIN, DEXTROSE, AND CYANIDE ON THE CIRCULAR AND THE LONGITUDINAL MUSCLES OF THE RABBIT'S INTESTINE

A THESIS
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INTRODUCTION

During the last few years, several papers have appeared in the literature dealing with the protective action of dextrose and insulin against hydrocyanic acid poisoning. Violle (1926), Kohn-Abreast and Lupu (1928), and Wiegand (1932) found that glucose would antagonize hydrocyanic acid poisoning. Heyman and Soenen (1927) reported a negative effect of glucose upon cyanogen poisoning. A protective action by insulin was reported by Szolnoki (1926) and Rosenberg (1926). Hosoda (1929) found that insulin would counteract the hyperglycemia caused by hydrocyanic acid injections. Puder (1927) and Forst (1928) stated that insulin in conjunction with dextrose would give complete protection against hydrocyanic acid under certain conditions.

As it had been found in previous work that the excised intestinal segments were rather sensitive to each of the three substances mentioned above, it was decided to determine whether or not the protective action of insulin and dextrose could be demonstrated by this method of study.
METHOD AND MATERIAL

The apparatus used in this study is that described by Ets and Valentine (1932) Figure I.

Figure I

It has been slightly modified so that the second lever, for both the circular and longitudinal record, instead of writing directly on the kymograph, is connected to a balanced writing point suspended inside a slotted brass tube. The advantage of this change is that the writing points move perpendicularly instead of in an arc. The apparatus now records contractions, of both the circular and longitudinal muscles, as downward movements of the writing points.

A modified Tyrode solution is used which varies from that given by Sollman and Hanzlik (1928) in that it contains a greater amount of sodium bicarbonate (one tenth per cent instead of fifteen thousandths per cent) and no sodium acid phosphate or glucose. The other constituents are sodium
chloride (eight tenths of a gram per cent), potassium chloride (two one hundredths of a gram per cent), calcium chloride (one one hundredth of a gram per cent) and magnesium chloride (one one hundredth of a gram per cent). This solution has a pH of about seven and four tenths which is maintained constant by the method of Thomas (1931) which consists of equilibrating the air bubbled through the bath with carbon dioxide at a definite tension. The air to be used is passed through two washing bottles containing powdered magnesium carbonate and solutions of phosphate buffers. Powdered magnesium carbonate, suspended in solutions of the alkali phosphates, decomposes so as to maintain a constant carbon dioxide tension in the mixture over a long period of time. Air or oxygen that is in equilibrium with such a mixture will, when bubbled through Locke's solution, establish and maintain a constant carbon dioxide tension and hence a constant pH in the solution.

The rabbits used were young healthy animals weighing about two kilograms. The diet consisted of alfalfa and oats with the addition, twice a week, of fresh cabbage. The animals, when used, had not been given food for at least eighteen hours. They were killed by a blow at the base of the skull. The ileum was removed and that portion which was not to be used immediately was preserved in a dry beaker in the ice box as recommended by Gunn and Underhill (1914).

In the upper right hand corner of Figure I is a diagram
showing how the intestinal segment is connected in the apparatus. First a small glass bead is tied into the proximal end of the segment which is about three inches long. This end is then attached to the movable lever. The distal end is slipped over the glass cannula and tied. This lever records the longitudinal movements. The cannula is connected to the leveling bulb and water manometer, and records the internal pressure or volume changes. The leveling bulb is adjusted so that the internal pressure is just sufficient to balance the two levers for recording the circular movements. The levers were arranged so that the pressure required was usually from four to six centimeters of water. They are set at right angles to the first lever and are about three centimeters apart.

The intestinal bath contains two hundred and fifty cubic centimeters of the solution described above and is kept at a constant temperature of $37^\circ$ by a water bath. The concentration of the drugs is figured on the basis of the amount of drug in this two hundred and fifty cubic centimeters.
RESULTS

The following results are an analysis of the records of the effects of the drugs used on the tone, amplitude, and rhythm of the circular and longitudinal muscle contractions and on variations of the internal pressure brought about by the intestinal activity. The tone is determined by measuring the distance, on the graphs, from the mid-point of the amplitude of the contraction to the base line. By amplitude is meant the height of the excursion of the writing point. Rhythm refers to the regularity of rate and character of the contractions.

It is difficult to determine in certain instances whether the recorded increase or decrease in tone is active or passive or a combination of both i.e., are the changes due to contraction of the longitudinal which shortens the segment and widens the lumen and relaxation which lengthens the segment and narrows the lumen as observed by Cowie and Lashmet (1929) or are they due to actual contraction and relaxation of the circular muscle or are they due to both?

1. The effects of insulin (five units per two hundred and fifty cubic centimeters of Locke's solution) on a fresh segment of the intestine were studied. The results are based on thirty-two experiments. Figures II and III show photographs of records of the two typical effects of insulin.
The most common effect is illustrated in Figure II which shows a very slight increase in the tone of the circular muscle, a decreased amplitude, and practically no change in the rhythm. The longitudinal shows a decrease in tone and amplitude and although Figure II shows an interference with the rhythm, this is not the most common effect as it occurs in only ten percent of the cases. In no instance was there any significant change in the tone of the internal pressure tracing. That shown in Figure II is as great as was observed and was exceptional. In over eighty per cent of the experiments, the rhythm was unaffected. In general, then, it is indicated that insulin decreases the longitudinal tone and increases or does not effect the circular tone while the amplitude of both is decreased and the rhythm unaffected. These results were observed in about seventy-five per cent of the experiments.
The second typical effect of insulin is shown in Figure III and was seen in a little over ten per cent of the experiments. The outstanding difference between this effect and that shown in Figure II is the marked improvement in rhythm which is shown especially in the circular muscle. In about fifteen per cent of the experiments, mixed effects were obtained i.e., a combination of the effects described in Figure II and Figure III.

2. The effects of dextrose in concentrations of one part in one thousand on forty-nine fresh segments of the intestine were studied. Figure IV is a photograph of a record showing the typical effect of dextrose.

Proximal Circular
Distal Circular
Internal Pressure
Longitudinal
Time Record
(15 sec. intervals) Figure IV

The circular muscle shows a slight decrease in tone, (not always seen) an increase in amplitude and no change in rhythm. The longitudinal muscle shows a marked increase in tone and amplitude and very little change in rhythm. There is no change in the internal pressure but the amplitude and the rhythm of pressure changes are improved. If the dextrose is administered to a segment which is already contracting strongly the stimulatory effects mentioned above are not observed or else they
are very slight. When, however, the contractions of the segment were weak from fatigue, the stimulatory effects of dextrose were obtained in over ninety-five per cent of the cases.

3. The effects of sodium cyanide (one part in one million) were studied on eighteen fresh segments of the intestine. The typical effects of sodium cyanide in this dose are shown in Figure V.

Proximal Circular  
Distal Circular  
Internal Pressure  
Longitudinal  
Time Record (15 sec. intervals)  

Figure V

The contractions of the circular muscle are completely inhibited, but a slight increase in tone is recorded. The contractions of the longitudinal muscle are also completely inhibited except for a few small irregular contractions and the tone is very definitely decreased. This inhibitory effect of sodium cyanide is very constant and was essentially the same in all the experiments, the only variation being in the degree of depression. There was complete inhibition of contractions of the circular muscle in seventy per cent of the experiments and of the longitudinal muscle in about forty per cent. In the remainder, the depression was very marked but not complete.
4. The effect of sodium cyanide (one part in one million) on a fresh segment of the intestine after the administration of insulin (five units per two hundred and fifty cubic centimeters of bath) were studied in 13 experiments. The effect of sodium cyanide after insulin is a decreased amplitude of both the circular and longitudinal muscles, a small decrease of the longitudinal tone and slight increase of the circular tone. The internal pressure record shows, usually, only a slight decrease in amplitude. Figure VI shows most of these effects.

Proximal Circular  
Distal Circular  
Internal Pressure  
Longitudinal  
Time Record  
(15 sec. intervals)  
Figure VI

The inhibition of the circular contractions, however, is not the most common effect as it was in the absence of insulin: it occurred in only forty per cent of the experiments. The longitudinal contractions were completely inhibited only once in ten trials. These results indicate that the intensity of the sodium cyanide action has been lessened by insulin.

5. The effect of sodium cyanide (one part in one million) on a fresh segment of the intestine after the administration of dextrose in concentration of one part in one thousand were studied in eight experiments. The effect of
sodium cyanide after dextrose is very much the same as after insulin.

Figure VII indicates more protection against the sodium cyanide than Figure VI, but it must be observed that the segment in Figure VII was contracting more powerfully than was the segment in Figure VI. Dextrose alone does, nevertheless, give a greater degree of protection than does insulin alone. The number of times that complete inhibition of the circular muscle was observed dropped to a little over thirty-five per cent and in no instance at all was complete inhibition of the longitudinal muscle observed although the amplitude of the contractions was decreased in over sixty per cent of the cases.

6. The effect of sodium cyanide (one part in one million) on a fresh segment of the intestine after the administration of insulin (five units per two hundred and fifty cubic centimeters of bath) and dextrose (one part in one thousand) were studied in thirty-one experiments. Figure VIII illustrates an instance of practically complete protection against the sodium cyanide.
There is, in fact, not only protection from cyanide, but an actual stimulation as shown by the increase in tone and amplitude of contractions of both the circular and longitudinal muscles. Effects such as these, however, are exceptional and were observed in only a little over ten per cent of the experiments. In the remainder of the experiments, some depressive effect from cyanide was observed, but it was not very marked. In none of the experiments after the administration of both insulin and dextrose did the cyanide cause a complete inhibition of the contractions of the circular or longitudinal muscles.
DISCUSSION

In 1924, Winter and Smith reported that insulin produced a transient diminution in the tonus and height of the contractions of the intestinal segment. Barlow (1931) stated that on the excised rabbit intestine insulin produced a brief depression, mainly on the tonus, followed by a more lasting stimulation. He believes that insulin has a direct stimulant action on the smooth muscle of the intestine. Pavel and Milco (1932) report that in the majority of their experiments insulin augmented the contractility of the intestine in vitro, but in a small number of cases insulin had either no effect or it caused an inhibition of the contractions. They believe that insulin acts to stimulate the terminations of the peripheral parasympathetics. The results of the present study on intestinal motility are in accordance with the observation that insulin causes an initial depression of the tone and amplitude of the contractions. This decrease in tone accounts for the recorded increase in circular tone according to the observation of Cowie and Lashmet (1929). The later effect of stimulation described by Barlow (1931) is not indicated perhaps because sufficient time was not allowed for these stimulatory effects to become pronounced.

The use of dextrose in irrigating fluids was first recommended by Locke in 1907. From his work and that of many others since it has been concluded that the addition of dex-
trose (one tenth of a gram per cent) adds considerably to the maintenance of functional activity in contractile tissue and is indispensable for the mammalian heart. In this study, dextrose was not used as a constituent of the irrigating fluid because it was desired to observe the effects that the addition of dextrose might have on the action of sodium cyanide. The results obtained indicate that the addition of dextrose (one part in one thousand) in practically all the experiments had either a definitely beneficial effect or had no effect at all.

Hydrocyanic acid is a general protoplasmic poison. Compounds containing the cyanide radical are toxic only if they can liberate hydrocyanic acid. That the action of this acid is not to cause an anoxemia but is a disturbance of the oxygen utilizing power of the cells was first suspected by Geppert (1889) who stated that the action of cyanide is an "internal asphyxia" of the organs in the presence of excess oxygen. The work of Warburg indicates that an iron containing pigment, related to hematin, is present in the tissues in minute concentrations and is the essential catalyst which is responsible for oxidation in the cells. He believes that hydrocyanic acid renders this catalyst inactive by uniting with it and forming a catalytically inactive compound. This is brought about by a specific chemical reaction Cytochrome (Keilin, 1929) is a pigment, the distribution of which is re-
lated to the activity of tissues. It exists in an oxidized and a reduced form. Oxidized cytochrome is rapidly reduced in the tissues if oxygen is excluded and in the presence of molecular oxygen reduced cytochrome is readily oxidized. If cells containing reduced cytochrome are treated with cyanide the pigment remains reduced and can no longer be oxidized by molecular oxygen. This indicates that the oxidation of cytochrome is catalyzed by a ferment made inactive by cyanide. This is a second example which relates the depression of oxidation brought about by cyanide to an inactivation of a tissue enzyme.

Alvarez (1918) working with excised rabbit intestine found that the addition of potassium cyanide gave results similar to those of shutting off the oxygen supply which was being bubbled through the solution. He reported that oxygen lack from either cause depressed the intestinal contractions, especially in the duodenum and jejunum which have a greater metabolic rate than the ileum.

In this study it was found that sodium cyanide (one part in one million) was the minimum dose which would cause complete inhibition or a marked depression of the contractions of the fresh segment. It was decided, therefore, to use this dose (one part in one million) as a standard and to compare the effects on a fresh segment with the effects on a segment after the administration of insulin and dextrose, separately and together.
In 1926, Szolnoki reported a protective action of insulin against hydrocyanic acid poisoning in rabbits. Similar results were obtained by Rosenberg (1926), also working with rabbits, who reported that insulin contained an oxidation-promoting component and would counteract hydrocyanic acid poisoning. He believes with Szolnoki that insulin deserves to be recognized as an antidote for hydrocyanic acid poisoning. Hosoda (1929) found that the injection of insulin lowered the hyperglycemia in rabbits, produced by an injection of hydrocyanic acid.

In the present work on isolated intestinal segments it was found that administration of insulin would protect, to some extent, against the action of sodium cyanide. The antagonistic effects are most evident on the amplitude of the contractions. When the sodium cyanide was given after the administration of insulin there were fewer complete inhibitions and the decreases in amplitude were not as great as when the cyanide was given on a fresh segment. The tone changes were also smaller when insulin was present.

In 1926, Violle found that if rabbits were given glucose and then transferred to an atmosphere containing hydrocyanic acid gas they could survive many times the lethal dose determined on control animals. Heyman and Soenen (1927) could not confirm these results. Their animals did not show any increased resistance to the inhalation of hydrocyanic acid.
Baker and DeLong (1927) in studying the habits and means of control of the onion root maggot made an extensive investigation of the chemotropic responses of the flies to various substances. They found that the toxicity of a sodium cyanide and molasses mixture was practically nil. The mixture gave off a distinct odor of ammonia. They believe that the most probable explanation of the production of ammonia in the mixture appears to be that of an interaction between sodium cyanide and the glucose of the molasses to yield glucose cyanhydrim, which subsequently hydrolyzes to form ammonia. When a cane sugar solution was substituted for the molasses hydrocyanic acid was evolved, usually sufficient to kill most of the flies. Forst (1928) reported that injections of glucose in white mice and white rats would protect against a four-fold lethal dose of hydrocyanic acid. The protection is believed to be due to the dihydroxyacetone formed from dextrose which antagonizes the hydrocyanic acid. Wiegand (1931) found that dihydroxyacetone and dextrose would remove the respiratory inhibition of potassium cyanide on the liver cells of mice in vitro. He attributes the protection to a combination of dihydroxyacetone with the cyanide thus preventing the cyanide from uniting with the cellular iron.

The results obtained on the excised intestine show that while there is a definite effect of the cyanide after the administration of dextrose it is by no means as pronounced as it is without the dextrose.
Since either dextrose or insulin give a certain degree of protection against sodium cyanide poisoning, the degree of protection afforded by the combined effects of both dextrose and insulin should be much more complete.

Puder (1927) working with Szolnoki and Erdös undertook an investigation to ascertain under what circumstances insulin and dextrose would give protection against hydrocyanic acid and upon what the protective action depended. The work was carried out on rabbits. For dextrose they found that four grams per kilogram of body weight must be given for each three milligrams per kilogram of body weight of hydrocyanic acid. They attribute this protection to the formation of nitrils. For insulin they found that twenty units would act as a protective dose if given ten minutes before a three milligram per kilogram dose of cyanide. Puder suggests three possibilities as the mechanism of action of insulin protection. The first possibility is that insulin acts as a stimulant and cyanide as an inhibitor of tissue respiration. Together they are to be considered as antagonists as regards tissue respiration. This is based on the observation that the inhibition of catalysis caused by cyanide is not as great if insulin is present as it is when cyanide alone is present. The second possibility is that insulin can in itself bind the cyanide containing radical. This was suggested by the work of Erdös who found that a compound could be precipitated from insulin by phenylhydrazine,
the nitrogen content of which indicates that insulin contains a constituent which will bind cyanide. The third possibility of the mode of action is that insulin produces dextrose from glycogen and through this the cyanide is bound in the form of a nitril.

Wiegand (1931) has studied the detoxification of hydrocyanic acid by dextrose and dihydroxyacetone and suggests that the action is through a chemical binding of the cyanide by the carbonyl group thus forming an α-hydroxyacid nitril as follows:

\[ R\text{-}C=O + HCN \rightarrow R\text{-}C\text{-}OH \]
\[ R\text{-}C\text{-}CN \]

This, however, is only a partial detoxification. Complete detoxification results when the α-hydroxyacid nitril is converted to a hydroxycarboxylic acid:

\[ R\text{-}C\text{-}OH + 2H_2O \rightarrow R\text{-}C\text{-}COOH + NH_3 \]

Wiegand believes that the importance of this latter reaction should be given more emphasis in the literature.

The protective action of insulin and dextrose against hydrocyanic acid poisoning was shown quite definitely on the isolated segment of the intestine. In no instance, after the administration of insulin and dextrose did the sodium cyanide completely inhibit the contractions and when a decrease of tone, amplitude, or rhythm was observed it was, in practically all cases, much smaller than that observed when the sodium cyanide was administered to a fresh segment.
SUMMARY AND CONCLUSIONS

1. Insulin decreases the tone and the amplitude of the contractions of the isolated segment of the rabbit's intestine.

2. Dextrose increases the tone and the amplitude of the contractions of the isolated segment or, if the segment is contracting strongly, it will show no effect at all.

3. Sodium cyanide will completely inhibit or very markedly decrease the tone and the contractions of the isolated segment.

4. The intensity of the action of sodium cyanide is diminished to a considerable degree by the administration of either dextrose or insulin before the administration of the sodium cyanide.

5. The administration of both insulin and dextrose before the sodium cyanide gave, in the majority of the experiments, practically complete protection against the sodium cyanide.
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