CXLVI. THE ANTAGONISM OF GLUCOSONE AND CYANIDES *IN VIVO*.

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Recently many references have appeared in the literature to the antagonistic action towards cyanides which results from the previous administration of glucose either alone, or in conjunction with insulin. As, in the course of experiments on the physiological action of glucosone [Hynd, 1927], it had been noticed that an antagonism also appeared to exist between glucosone and potassium cyanide, the question was investigated more fully in the hope of correlating the results referred to above.

Owing to the small amount of glucosone available, the majority of the experiments involving the use of this compound were carried out on mice, but a certain number were performed on young albino rats and also on rabbits. In the experiments described 67 mice, 14 rats and 14 rabbits have been employed.

I. The subcutaneous injection of potassium cyanide into mice.

In the first experiments potassium cyanide was administered subcutaneously. The mice used were found to be much more resistant to this poison than was expected, and consequently a number of preliminary trials had to be made to determine the necessary dosage for the object in view. From these it appeared that 4 minims of a 0·5 % solution of potassium cyanide was a suitable dose for a mouse weighing 20 g. Such a dose of cyanide rarely renders a mouse moribund, but nevertheless it produces, in a short time, very definite symptoms, usually the first to be noted being a slight retraction of the head and "biting at the air." Within five minutes after the injection leg-weakness is noticeable, and this soon becomes more marked so that in the course of another five minutes the animal is unable to walk—any attempt to do so being followed by severe tremors. Handling of the mouse at this stage also induces tremors, after which the animal sometimes rolls over and lies on its back. Return to the prone position may not occur for some time, but it usually takes place suddenly, and is sometimes accompanied by a convulsion. Thereafter recovery takes place slowly and the mouse appears fairly normal in about two hours after the injection.

Three mice of approximately equal weight were selected for each experiment—the first being injected with cyanide, the second with a suitable amount of glucosone, and the third receiving injections of the same amounts of both cyanide and glucosone.

An 11·35 % solution of glucosone was used and 0·3 of a minim per g. body-weight was the amount injected, as such a dose was known to be non-lethal but capable of producing severe symptoms. This always was realised in the animals receiving glucosone alone—marked sprawling of the legs being evident in 5 to 10 minutes after injection and more severe symptoms in the course of 15 to 20 minutes.

Animals receiving injections of both glucosone and cyanide were from 20 to 30 minutes before exhibiting any symptoms. Usually a slight glucosone effect, such as sprawling of the legs, was observed 20 minutes after the injection instead of in 5 minutes in the control animals, and no cyanide effect was detected till 30 minutes had elapsed. The symptoms displayed were of a mild character and of a mixed type, at one moment suggesting the cyanide, at another the glucosone action, but return to the normal required a somewhat longer period than was the case with a mouse receiving potassium cyanide alone.

This experiment has been repeated on three separate sets of animals with uniform results.

II. The subcutaneous injection of acetonitrile.

A series of experiments was then carried out in which the alkali cyanide was replaced by acetonitrile. As the result of preliminary experiments it was found that a dose of 0·2 minim of a 5 % solution of acetonitrile per g. of body-weight produced in a mouse a fairly characteristic train of symptoms. These included tremors, lowering of body temperature, sprawling limbs and convulsions.

As before, three mice of approximately the same weight were used in each experiment—the first being injected with acetonitrile, the second with acetonitrile and glucose, and the third with acetonitrile and glucosone. All the animals used in this set of experiments exhibited in about 20 minutes marked sprawling of both fore and hind limbs, while at a later stage those not receiving glucosone, as a rule, rolled over on their backs and lay clasping the fore limbs together. The "glucosoned" mice showed no sign of tremors, which was a striking feature in the other animals. Moreover, though on some occasions, half an hour after being injected, the mice which had received glucosone appeared most affected, after 3 to 4 hours they were quite normal, whereas the others still exhibited effects of the nitrile (including diarrhoea) and, in some cases, the mice injected with acetonitrile alone were found dead the following morning.

III. The effect of an atmosphere containing hydrogen cyanide.

The effect of the inhalation of hydrogen cyanide was then studied on mice. For this purpose a bell-jar of 7 l. capacity, closed with a ground glass plate and fitted with a hydrogen cyanide generator, was used—the latter being so arranged that the hydrocyanic acid was liberated only after the experimental animals had been introduced into the bell-jar. By trial it was found that the gas evolved from the interaction of 3 cc. of 4 % potassium cyanide and 2 cc. of 40 % sulphuric acid, furnished a suitable concentration of hydrogen cyanide. Although the exact concentration of the hydrogen cyanide was unknown, this was immaterial, as three animals were used in each experiment—the first injected with a non-lethal dose of glucosone, the second with an equivalent amount of glucose, and the third not injected. Animals of equal weight were chosen as far as possible, and where this could not be managed, the lightest received glucosone and the heaviest was used as the normal animal. Eight experiments of this type have been carried out and invariably it has been found that glucose affords no protection against hydrogen cyanide. On the other hand, a subcutaneous injection of glucosone enables a mouse to resist the toxic action of hydrogen cyanide. These facts are clearly demonstrated in the following summary of a more or less typical experiment.

Effect of an atmosphere containing hydrogen cyanide.

Time a.m.	Black mouse 20·0 g.	White mouse 19·4 g.	Grey and white mouse 19·6 g.
10.9	No injection	3 minims 18·13 % glucosone	3 minims 20 % glucose
10.15	HCN atmosphere	HCN atmosphere	HCN atmosphere
10.16	Running about sniffing	Sitting with head down	Appears normal
10.19	Respiration very rapid: beginning of cyanide effect	Slight sprawling of legs: due to glucosone	Appears drowsy
10.24	Leaping about	Slight sprawling of legs: due to glucosone	Walking about
10.28	Sitting gasping: still able to cling	Still sprawling but can walk about when disturbed	Beginning to show effect of cyanide
10.30	Lying on side: gasping	Slightly improved but eyes protruding	Violent convulsion: then rolls over on to its side
10.32	Symptoms very severe: slight convulsion	Slightly improved but eyes protruding	Respirations very slow
10.34	Rolls over: several severe convulsions	Slightly improved but eyes protruding	Eyes protruding
10.52	Lying moribund	Beginning to show slight cyanide effect	Dead
	Removed to ordinary air.	*Removed to ordinary air.*	
10.56	Severe convulsions	Sitting in normal position but unwilling to move	
11.0	Slightly improved	Definitely improved	
11.5	Still blind: severe tremors when handled	No tremors, but signs of diarrhoea	
11.10	Several convulsions	Fairly normal	
11.30	Improving generally: eyes normal	Fairly normal	
p.m. 2.0	Practically normal	Drowsy: fur ruffled	

Certain of the experiments carried out have been even more striking than the above, as, for example, that in which a normal mouse of 26 g. weight was rendered moribund in 10 minutes by the hydrogen cyanide vapour, while a 20 g. mouse, which had been injected with glucosone, showed only slight symptoms after 2½ hours' inhalation, at which time the normal mouse died.

Very similar results have been obtained in three experiments with young albino rats of an average weight of 35 g. and from the same litter, and also in one experiment when the average weight was 75 g. All the rats used in these experiments survived, but the striking feature was the rapidity with which those injected with glucosone became normal on removal from the atmosphere of hydrogen cyanide. The recoveries of both the "normal" rat and that injected with glucose were in comparison slow, and moreover were accompanied by a number of severe convulsions.

A number of experiments were also carried out to determine whether insulin with or without glucose furnished any protection for mice exposed to the vapour of hydrogen cyanide. Doses of insulin varying from 0·8 to 1·6 units per 100 g. body-weight were employed, but no definite protective action could be demonstrated. In fact all the insulinised mice, which were exposed to gaseous hydrocyanic acid, died, while normal mice, though displaying severe cyanide symptoms as the result of the same exposure, recovered on removal to ordinary air. In the majority of the experiments it appeared that exposure to the atmosphere of hydrogen cyanide hastened the development of the insulin symptoms, but this point could not be established by control experiments.

IV. Experiments involving the intravenous injection of potassium cyanide into rabbits.

(i) *The antagonistic action of insulin plus glucose.*

(*a*) A rabbit of 2·7 kg. was injected intravenously with 30 units of insulin and 4 cc. of a 40 % solution of glucose. Eight minutes later, 5 minims of a 4 % solution of potassium cyanide were injected into the same ear vein. Immediately the animal showed difficulty in breathing and, as soon as released, it rolled over on its side. The head was retracted and respirations were very slow, but in 2 to 3 minutes they improved slightly. This was followed a minute later by a severe convulsion, and two minutes afterwards the animal was able to sit up, although the hind limbs were still extended. Ten minutes later the rabbit looked quite normal and no further symptoms were noticed during the course of the day.

Two days later, using the same rabbit, the experiment was repeated except that no insulin was administered. The animal behaved exactly as on the previous occasion—severe symptoms immediately after the cyanide injection and return to the normal in about 10 minutes.

(*b*) The above experiment was repeated with a larger dose of cyanide (8 minims of 4 % KCN) using two rabbits weighing 2720 g. and 2940 g.

respectively—the former, a male, receiving 30 units of insulin and the latter, a female, no insulin. They behaved in exactly the same manner, except that after returning apparently to the normal condition the insulinised male rabbit showed slight insulin symptoms but recovered without any treatment.

(*c*) Having found that the intravenous injection of 5 minims 10 % potassium cyanide into a rabbit (♂) weighing 3 kg. caused death almost instantaneously, another rabbit (♀) of the same weight was injected intravenously with 4 cc. of 40 % glucose and 30 units of insulin, and five minutes later 5 minims 10 % potassium cyanide were introduced into the marginal vein of the other ear. Severe symptoms ensued immediately, but 20 minutes later the animal was fairly normal, its behaviour having been very similar to that of the first rabbit described above in experiment (*a*).

Five days later this experiment was repeated on the same animal with identical results. After another period of five days the same rabbit was used to study the effect of the same amount of cyanide after glucose but without insulin. Complete though somewhat slow recovery took place, the rabbit being able to sit up in the normal position 45 minutes after the injection. About a fortnight later, on injecting the same amount of cyanide alone, this rabbit collapsed at once and died in one and a half minutes.

(ii) *The antagonistic action of glucosone.*

(*a*) A young rabbit weighing 900 g. was injected intravenously with 1·5 cc. of 18·13 % glucosone[1], and then immediately afterwards with 3 minims of 5 % potassium cyanide. Severe struggling took place for about half a minute, thereafter the animal lay on its side quite collapsed. After one minute the corneal reflex returned. No twitching or tremors were noticed, but the rabbit lay quite limp for about ten minutes, at which time it was able to hold up its head, although the limbs remained limp till about five minutes later. Twenty minutes after receiving the injection, the animal appeared drowsy but otherwise normal.

A week later the effect of the same dose of cyanide was studied on the same rabbit. As before severe struggling took place, followed by collapse, the animal lying on its side helpless, with head retracted and eyes fixed. Respirations, which at first were slow, gradually improved in the course of 2 to 3 minutes, during which time a number of inspiratory cries were uttered. Five minutes elapsed before the reflexes returned: in ten minutes, after marked twitching especially of the fore limbs, an attempt was made to sit up, but after doing so the rabbit gradually fell over again on its side. Two minutes later, however, it succeeded in resuming the normal position but was not quite recovered in half an hour, being disinclined to move, sitting with ruffled fur and having a general scared appearance.

[1] So far the physiological action of glucosone has been reported only on mice [Hynd, 1927]. Its action on other species has been investigated and an account of this is at present in preparation.

(*b*) Two young rabbits of the same litter and of approximately the same weight were used for the next experiment. One (530 g.), on injection with 1·6 minims of 5 % sodium cyanide, displayed the usual symptoms and died in 1½ minutes. The other (550 g.) was given intravenously 1 cc. of 18·13 % glucosone and five minutes later 1·6 minims of 5 % sodium cyanide. Less severe struggling ensued and the animal died within a minute of receiving the cyanide.

(*c*) In the following experiment the dosage of cyanide was reduced by half, and two rabbits of the same weight (620 g.) and from the same litter were employed.

20 minims tap-water mixed with 1·7 minims 2·5 % sodium cyanide were injected into the left ear vein of one animal. After half a minute's violent struggling the rabbit lay collapsed on its side. Improvement in respiration was noted in three minutes, but reflexes did not return for 11 minutes. Efforts to hold the head erect were not made until 18 minutes had elapsed, and at this time both fore and hind limbs were still sprawling. Return to a fairly normal condition required about 50 minutes.

20 minims 18·13 % glucosone mixed with 1·7 minims 2·5 % sodium cyanide were similarly injected into the other rabbit. Struggling was not nearly so marked and the animal never collapsed. Reflexes were never absent, the head was kept always erect, but the limbs were extended for a few minutes. Three minutes after the injection the rabbit seemed fairly normal but looked frightened.

Two days later the treatment of the animals was reversed. On giving the same dose of glucosone and cyanide to the first rabbit slight struggling occurred during the injection but otherwise no effect was noticed. The second rabbit, on the other hand, struggled so violently during the injection of cyanide alone that only half the amount was introduced and the animal died within a minute.

Three days later, similar behaviour occurred with the surviving rabbit when it was injected with 1·7 minims of 2·5 % sodium cyanide previously diluted with 18 minims of tap-water. After very violent struggling during the injection, the animal collapsed and died almost instantaneously.

Discussion.

The view expressed by Horvath [1926] that the therapeutic value of glucose consists in part in an antitoxic rôle toward the CN-group, is not borne out by the results of the experiments now described. In no case has there been observed any definite protective action of glucose against alkali cyanide, gaseous hydrocyanic acid, or acetonitrile. This is also contrary to the findings of Violle [1926], who has stated that, while a concentration of 2 g. hydrogen cyanide per cubic metre of air will kill a rabbit in a few minutes, animals previously fed or injected with glucose can breathe this toxic atmosphere for more than an hour without ill effects. As the concentration of hydrogen

cyanide used in his experiments was of the same order as that employed by the present author, there is the possibility that the divergence in the results might be explained by the fact that the experiments were conducted on a different species of animal, for, while Violle used rabbits, my comparable experiments were confined to mice and rats. This explanation, however, seems unlikely as already Voegtlin, Johnson and Dyer [1926] and also Heymans and Soenen [1927], who used rabbits and guinea-pigs, have described experiments, the results of which point to glucose being incapable of counteracting the toxic effects of cyanogen compounds.

On the other hand, the results of the present investigation point to the fact that glucosone and potassium cyanide are antagonistic in their actions in the animal organism, for the symptoms accruing from either compound are delayed in their development and diminished in their intensity when simultaneous injections of these substances are given subcutaneously.

Evidence that glucosone counteracts the CN-group is also furnished by the experiments with acetonitrile. These were performed in the hope that this somewhat less toxic compound would yield even more definite results than those obtained with the alkali cyanide. This was not realised, however, and, in fact, the results were somewhat less definite.

Although controls were always carried out against experiments involving the subcutaneous injection of cyanides, it was difficult, especially when dealing with small animals such as mice, to be certain that the proper amount was injected, and even though this operation had been accomplished successfully, there was no guarantee that the rate of absorption would be comparable in the experimental and the control animal. However, any objection on this account is negatived owing to the number of experiments carried out and the uniformity of the results obtained.

Further evidence of a convincing character, as to the antagonistic action of glucosone and cyanide, is furnished by the results of the experiments in which the experimental animals were exposed to an atmosphere containing hydrogen cyanide. Though very definite results were obtained in these inhalation experiments with both mice and rats, the dose of glucosone required to afford protection is a fairly large one, being only slightly less than what would be a lethal dose under normal atmospheric conditions. This may explain why insulin with or without glucose, or even glucose itself, has been found to be useless for small animals, as sufficient glucosone would not be produced quickly enough to counteract the large amount of cyanide. The author's suggestion [Hynd, 1927] that insulin converts inactive glucose into utilisable glucosone, may provide the key for the explanation of the results reported by Szolnoki [1926] and by Rosenberg [1926] regarding the antidotal action of insulin in hydrocyanic acid poisoning.

Reference to the experimental part dealing with the intravenous injection of potassium cyanide into rabbits will show that attempts to repeat the experiments described by Rosenberg [1926] were not altogether successful, as in the

third experiment the rabbit recovered from the cyanide injection when protected by glucose without insulin. The symptoms which ensue after an intravenous injection into a rabbit of an amount of cyanide approaching the lethal dose are so severe that recovery of the animal appears to be always rather problematic, no matter what substance has been administered for its protection. So large a number of factors are involved, as for example, the rate at which the cyanide is injected and its subsequent distribution, that one must hesitate in drawing too definite conclusions.

Nevertheless, taking the results of the present experiments as a whole, it seems established that glucosone exerts an antagonistic action towards the CN-group. In this respect, however, glucosone is not so efficient as, for example, cystine, with which it was compared and which, as Voègtlin and his co-workers have shown, is able to protect rats against lethal doses of cyanide. Consequently, glucosone in no sense can be recommended as an antidote in hydrocyanic acid poisoning, and evidently the use of glucose or of glucose plus insulin would be of even less avail for such a purpose.

It is now generally accepted that the normal mechanism of detoxicating cyanides in the animal body involves interaction with sulphur compounds. Although this may be true under normal conditions, it seems possible that, when excessive amounts of cyanides have to be dealt with, the supply of such thio-compounds may be inadequate and carbohydrate may then be called upon, just as glycuronates appear in the urine when there is produced an excess of compounds which normally are eliminated as ethereal sulphates. As in glucosone, an oxidation product of glucose, the body would have at its command an efficient detoxicating agent, it seems possible, in view of the results already obtained [Hynd, 1927], that glucosone is a compound of real physiological importance.

Summary.

1. In the case of mice and rats, a subcutaneous injection of glucose, or of glucose plus insulin, affords no protection against the toxic action following (*a*) the subsequent subcutaneous injection of either alkali or methyl cyanides, or (*b*) the inhalation of gaseous hydrogen cyanide.

2. Under the same experimental conditions, glucosone exerts a definite antagonistic action towards cyanides, but is less efficient than cystine.

3. The previous, or simultaneous, intravenous administration of glucosone, or of glucose plus insulin, appears to be protective against the intravenous injection of alkali cyanide into rabbits, but the results obtained are somewhat variable.

References.

Heymans and Soenen (1927). *Compt. Rend. Soc. Biol.* **96**, 202.
Horvath (1926). *Japan Med. World*, **6**, 133.
Hynd (1927). *Proc. Roy. Soc. Lond.* B, **101**, 244.
Rosenberg (1926). *Med. Klin. Berlin*, **22**, 1650.
Szolnoki (1926). *Deutsch. med. Woch.* **52**, 1427.
Violle (1926). *Bull. Acad. Med.* **95**, 664.
Voegtlin, Johnson and Dyer (1926). *J. Pharm. Exp. Ther.* **27**, 467.