CXVIII. GUANIDINE STUDIES.

IV. CHANGES IN GUANIDINE ACTION AND IN PARATHYROIDISM TETANY PRODUCED BY DEXTROSE.

By MAX MAPES ELLIS.

From the Physiological Institute of the University of Glasgow.

(Received June 6th, 1928.)

GUANIDINE acting as a strong base is known to form compounds with several sugars including dextrose and galactose [Herzfeld, 1895; Morrell and Bellars, 1907], and this fact, together with the recovery of relatively large quantities of guanidine from the livers of animals in guanidine tetany [Ellis, 1928, 1], suggested the possibility that sugar may aid in the disposal of guanidines in the body. Some chemical and physiological tests of dextrose-guanidine have, therefore, been made and the effects of combined injections of guanidine carbonate and dextrose compared with those of guanidine carbonate alone.

The dextrose-guanidine was prepared by combining a saturated solution of dextrose in absolute alcohol with a strong solution of guanidine in absolute alcohol, the precipitated dextrose-guanidine being centrifuged down after the mixture had stood for 40 hours in an ice-box [Morrell and Bellars, 1907]. The guanidine solution was obtained by adding to an aqueous solution of guanidine carbonate slightly less than the amount of barium hydroxide required to precipitate completely the carbonate from the guanidine carbonate, after which the barium carbonate was filtered off and the filtrate evaporated to a very thick syrup (almost to dryness) under reduced pressure at 40°. This syrup was taken up in absolute alcohol and filtered to remove guanidine carbonate, the filtrate dropping directly into the saturated solution of dextrose in absolute alcohol. It is essential that absolute alcohol be used both to take up the guanidine and to dissolve the dextrose, and that all operations be performed in closed vessels so that moisture and carbon dioxide may be excluded. With these precautions the yield of dextrose-guanidine is very good. After the dextrose-guanidine had been centrifuged down it was washed with fresh absolute alcohol, recentrifuged, washed with dry ether, and then dried in a vacuum desiccator. The dextrose-guanidine was white in colour, very hygroscopic, and gave a clear solution in water with just a faint trace of yellowish-brown colour.

Morrell and Bellars [1907] assigned the formula $3C_6H_{12}O_6 \cdot 2CH_5N_3$ to dextrose-guanidine and found it stable below 50° . They also noted that solutions of this substance are mutarotatory and that the rotation decreased during the first few days after the dextrose-guanidine was prepared, even if it were kept as dry powder, although it did not change in composition as determined by analysis.

A series of colorimetric determinations was made to ascertain the amounts of guanidine base in various quantities of dextrose-guanidine capable of giving the nitroprusside reaction. For these tests the various quantities of dry dextrose-guanidine were each taken up in 5 cc. of distilled water, the colour reaction obtained by means of the Marston [1924] reagent as modified by Weber [1927], and the readings made against known quantities of guanidine carbonate and guanidine hydrochloride. The results are summarised in Table I. The determinations show first of all that only a small portion of the guanidine base entering into the composition of dextrose-guanidine is capable of reacting with the nitroprusside reagent, which is very sensitive to minute quantities of guanidine salts and does not react with dextrose. The amount of dextrose-guanidine reacting as guanidine base is not proportional to the amount of dextrose-guanidine in solution, for the more dilute solutions gave relatively higher guanidine values. The age of the preparation was also a factor, for, after 24 hours in the desiccator as a dry powder, the portion of the dextrose-guanidine reacting with the nitroprusside reagent was still smaller. As controls against the guanidine determinations, samples containing 117 mg. of dextrose-guanidine per 100 cc. of distilled water were analysed for dextrose by the Hagedorn-Jensen method [1923] simultaneously with the first series of guanidine determinations; *i.e.* the sugar determinations are comparable with the first series of entries in Table I, and it was found that only about 57 % of the theoretical amount of dextrose in the dextrose-guanidine samples was determined by the Hagedorn-Jensen method. Dextrose-guanidine samples gave negative results when tested with the creatinine reagents of Folin [1922].

As the chemical tests indicated that guanidine did not react normally when combined with dextrose, a comparison of the toxicity of dextrose-guanidine, guanidine carbonate and guanidine hydrochloride was made. Six frogs of approximately equal weight were selected and placed in individual glass jars so that the factors of light, temperature and moisture were the same for all. After an hour for readjustment the animals were injected in the dorsal lymph sac, two with guanidine hydrochloride, two with guanidine carbonate and two with dextrose-guanidine, care being taken to disturb the animals as little as possible. They were then kept under continuous observation and the appearance of the several symptoms of guanidine poisoning recorded. Three points in the progress of the guanidine poisoning were noted in particular, the onset of continuous muscular tremor, the time at which the animal became prostrate and was unable to right itself after tetany spasms, and cessation of respiratory movements. The data from these animals (Table II) show that the dextrose-

guanidine used was much less toxic, weight for weight, than either guanidine carbonate or guanidine hydrochloride, and considerably less toxic than either of these two salts on the basis of the content of guanidine.

Table I. *Determinations of guanidine base in dextrose-guanidine samples.*

Immediately after drying				After 24 hours in desiccator as dry powder			
Dextrose-guanidine		Guanidine base		Dextrose-guanidine		Guanidine base	
Amount used (mg.)	Contained guanidine base* (mg.)	Amount found (mg.)	(%)	Amount used (mg.)	Contained guanidine base* (mg.)	Amount found (mg.)	(%)
1.17	0.210	0.079	37.8	1.18	0.211	0.046	21.8
2.34	0.420	0.144	34.3	2.35	0.421	0.062	14.8
3.51	0.630	0.195	31.0	4.70	0.842	0.046	5.5
				7.05	1.264	0.072	5.7
				11.75	2.106	0.084	4.0

* Computed from the molecular formula for dextrose-guanidine as given by Morrell and Bellars [1907].

Table II. *Comparisons of toxicity of dextrose-guanidine with guanidine carbonate and guanidine hydrochloride.*

Frog no.	Body-weight (g.)	Substance injected	Guanidine base equivalent (mg.)	Time after injection		
				Onset of tremors hrs. mins.	Prostration hrs. mins.	Cessation of respiration hrs. mins.
1	24.5	15 mg. guan. hydrochlor.	7.8	1 26	3 06	5 31
4	31.5	15 mg. guan. carb.	9.84	0 55	1 00	4 30
5	26.5	30 mg. guan. hydrochlor.	15.6	0 25	3 05	4 35
3	26.5	100 mg. dextrose-guanidine	17.94	4 00	6 15	Recovered
2	22.0	30 mg. guan. carb.	19.67	0 20	0 46	3 40
6	26.5	200 mg. dextrose-guanidine	35.88	1 06	3 10	5 45

All injections were 1 cc. in volume. The dextrose-guanidine used had been kept as a dry powder for 48 hours in a desiccator.

Since no method was available by means of which the presence of dextrose-guanidine as such could be demonstrated in living animals, and as the real value to an animal of such a sugar-guanidine combination with reduced toxicity would lie in the formation of this combination within the body from more active guanidine compounds, combined injections of dextrose and guanidine carbonate were compared with injections of guanidine carbonate alone. In Table III the condensed data from a series of ten frogs are given. To allow for differences in the rates of absorption of the dextrose and the guanidine carbonate, the dextrose was injected 1 hour in advance of the guanidine in two frogs, $\frac{1}{2}$ hour in advance in two, and simultaneously with the guanidine in two others. Large doses of dextrose were used to provide a liberal excess of dextrose above that required on a chemical basis to combine with the guanidine, if this combination were to take place within the body. As frogs will excrete dextrose very rapidly if kept in water (in another series of experiments the writer has found that 25 to 30 g. frogs will survive the injection of 1 to 1.5 g. of dextrose if placed at once in a large volume of fresh water), the frogs in this

guanidine and dextrose series were kept in individual glass jars containing only single moist filter-papers for the first 6 hours after the injections, to insure the presence in the body of a considerable portion of the injected dextrose during the period in which the guanidine poisoning symptoms should appear. The prompt appearance of the guanidine symptoms in the frogs which were given guanidine alone is in sharp contrast with the absence of guanidine symptoms during the first 5 hours in all frogs receiving guanidine and dextrose. With the exceptions of frog no. 18 which received its guanidine and dextrose simultaneously, and frog no. 15 which died 3 hours after the injection of guanidine, the other frogs receiving both dextrose and guanidine did not develop guanidine symptoms until they had remained in water for 12 hours or

Table III. *Comparison of dextrose and guanidine injections with injections of guanidine alone.*

Frog no.	Body-weight (g.)	Dextrose*		Guanidine carbonate† (mg.)	Time after injection		Final condition
		Amount (mg.)	Time		Onset of tremors (hrs. mins.)	Prostration (hrs. mins.)	
13	29.0	400	1 hour before guanidine	10	26 30	None	Recovery at 72 hours
14	25.5	200	1 hour before guanidine	10	48 00	None	Recovery at 72 hours
15	30.2	400	$\frac{1}{2}$ hour before guanidine	10	None	None	Dead, 3 hours 15 minutes
16	24.0	200	$\frac{1}{2}$ hour before guanidine	10	48 00	None	Recovery at 72 hours
17	31.7	400	With guanidine	10	48 00	None	Recovery at 72 hours
18	28.5	200	With guanidine	10	5 00	None	Dead, 48 hours
19	32.0	None	—	10‡	0 23	0 24	Recovery at 72 hours
20	36.7	None	—	10‡	0 22	1 38	Dead, 18 hours
21	23.5	200‡	—	None	None	None	Recovery at 72 hours
22	28.7	400‡	—	None	None	24 00	Dead, 27 hours

* Injected in 1 cc. of distilled water.

† Injected in 0.5 cc. of distilled water.

‡ Injected in 1.5 cc. of distilled water so that the total volume of fluid received by these animals would be the same as that received by the animals given two injections.

more, *i.e.* for 18 hours or more after the injection of the guanidine. Even frog no. 15 which presumably died from an overdose of dextrose (judging from the edematous condition of the body similar to that of other frogs killed with dextrose alone, *e.g.* frog no. 22) gave no evidence externally at least of guanidine poisoning. When placed in water the frogs which had received both guanidine and dextrose did develop guanidine tremors after 24 hours or more (see Table III) but without prostration, the tremors being much less severe than those in the frogs receiving only guanidine. In view of other experiments with dextrose alone it seems that the frogs which received both guanidine and dextrose lost dextrose rapidly, even after 6 hours in moist air, on being returned to water, and that the elimination of dextrose proceeded more rapidly than the elimination or destruction of the guanidine compounds. The protective value of the sugar against guanidine before the frogs were returned to water was evident however, and this was the point in question.

The mechanism of this relation between guanidine and dextrose in the body was not worked out in these preliminary experiments, but the relatively low toxicity of dextrose-guanidine offers an explanation if such sugar-guanidine compounds be formed in the body. It is also possible in the case of the frogs that changes in water balance may have been effected by the dextrose which offset those induced by guanidine poisoning and that the guanidine effects were counteracted in this way [see previous paper, Ellis, 1928, 2], for the muscles of frog no. 15 did not show an increase in the water content following the injection of dextrose and guanidine as did the muscles of frogs receiving guanidine alone. The work of Henderson [1910] must also be considered in this connection as presenting still another possibility. He found that the contractions and twitchings of isolated frog muscles caused by guanidine salts and by sodium oxalate were inhibited or prevented by immersing these muscles in solutions of glucose, mannitol and cane sugar, and suggested that the movement of the guanidine and the sodium oxalate into the muscle may be influenced by these sugar solutions.

As Paton and Findlay [1916] have favoured the view that parathyroprivia tetany results from an increase in the tissue-guanidines, the action of dextrose injections on parathyroidectomised cats was tried. Four parathyroidectomised cats were used, and each animal was kept until definite parathyroprivia tetany developed (usually on the third day after operation) before being injected. When the tetany became acute the first animal was given 20 units of insulin to lower the available sugar. This animal died in severe convulsions during the next 12 hours. The second and third parathyroidectomised cats were each given 10 cc. of sterile dextrose solution (20 %) intraperitoneally when the tetany became acute, and in each case the tetany disappeared and the animal showed marked improvement in condition during the following 6 to 8 hours. By repeating these injections once or twice a day these cats were kept free from tetany and in fair condition for 5 days, when the injections were terminated and the animals killed for analysis after being allowed to develop acute tetany again. The fourth cat was given an injection of adrenaline (1 cc. of 1 : 10,000) when the acute tetany stage was reached, and after an hour of marked depression the tetany disappeared and the condition of the animal was very good for the following 10 hours. The symptoms of tetany then gradually reappeared and the animal became prostrate again. A double injection of dextrose (20 cc.) was given and the animal recovered a second time. By alternating the adrenaline and dextrose injections this animal was kept free from tetany and in fair condition for 5 days, when it was allowed to develop acute tetany and was killed for analysis.

These tests on parathyroidectomised animals collectively favour the view that dextrose and guanidine are antagonistic, if guanidine be considered the causative agent for parathyroprivia tetany. If not, the correlation between the condition of tetany and the sugar level still remains, for the tetany became more acute when the sugar level was lowered by insulin, while the tetany was

in a large measure controlled by injections of dextrose or adrenaline, which raised the sugar level and maintained the animals for 3 or 4 days beyond the time at which uncontrolled parathyroidectomised animals usually die. It is interesting to add that Dr A. Watson, who has made certain histological studies of tissues from parathyroidectomised animals, informs me that the livers from animals in uncontrolled parathyroprivia tetany contained much glycogen.

SUMMARY.

1. Dextrose-guanidine was found to be less active both chemically and biologically than its equivalent of guanidine base.
2. Combined injections of dextrose and guanidine carbonate were less toxic than injections of guanidine carbonate alone.
3. By means of dextrose and adrenaline injections parathyroprivia tetany in cats was relieved, and these animals were maintained for 3 or 4 days beyond the time at which uncontrolled parathyroidectomised cats usually died.

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