THE USE OF BLOOD CHARCOAL AS A CLEARING AGENT FOR URINE CONTAINING GLUCOSE*

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In the course of some experiments on the quantitative excretion of sugars after parenteral injection, we had occasion to use Bang's[1] method for the determination of glucose and experienced, as have others who have used this method, the difficulty of determining sharply the end point of the reaction when dealing with urines that were even moderately colored. This difficulty is obviated by clearing the urine before titration and, according to Bang and Bohmannsson,[2] this clearing is readily accomplished without the loss of any appreciable amount of glucose by shaking the urine with blood charcoal after having rendered the urine 5 per cent. acid by means of hydrochloric acid. The loss as indicated by Bang and Bohmannsson under these conditions is so small that it can be neglected. Our first control experiment, however, showed quite the contrary, and further determinations indicated losses that amounted to 61 per cent. as determined by the polariscope and 43 per cent. as determined by the Bang method. Such gross variations were impossible to overlook, and could scarcely have been encountered by Bang and Bohmannsson without mention. Further examinations were therefore made to ascertain the source of this discrepancy.

We selected six normal urines which were clear enough to permit accurate polariscopic readings. These samples were made saccharine by the addition of pure dry glucose. To eliminate the error due to any optical changes which might occur as a result of the action of the acid or clearing agents on other urinary constituents besides sugar, these normal specimens were examined with the polariscope before and after clearing prior to the addition of sugar. In some instances the change in the reading was negligible, in others considerable. One specimen, for example, having before clearing a rotation of minus 9 minutes in a 20 cm. tube, showed plus 3 minutes after acid clearing. Corrections for these variations were made in the readings which were obtained from the same urines after glucose had been added. Small losses of glucose might other-

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1. Bang, I.: Zur Methodik der Zuckerbestimmung, Biochem. Ztschr. 1906-1907, ii, 271.

2. Bang, I., and Bohmannsson, G.: Zur Methodik der Harnzuckerbestimmung, Ztschr. f. physiol. Chem., 1909, lxiii, 443.

EXPERIMENTS ON THE USE OF BLOOD CHARCOAL AS A CLEARING AGENT FOR URINE CONTAINING GLUCOSE

	ROTATION						TITRATION		
Number of experiment	Normal urine	Normal urine cleared	Normal urine +glucose	Normal urine +glucose corrected	Normal urine +glucose cleared*	Normal urine +glucose cleared* corrected	Loss of glucose per cent.	Loss of glucose per cent.	Remarks
1	—0° 9′	+0° 3′	+1° 31′	+1° 40′	+1° 24′	+1° 21′	16	..	.....
2	—1° 6′	+0° 4′	+1° 38′	+1° 40′	+1° 22′	1° 18′	21	..	.....
3	—0° 4′	0° 0′	+1° 36′	+1° 40′	+1° 32′	1° 32′	8	..	.....
4	—0° 6′	+0° 1′	+1° 38′	+1° 38′	+1° 37′	1° 36′	2	..	.....
5	+0° 1′	+0° 3′	+1° 40′	+1° 39′	+1° 23′	1° 20′	20	..	.....
6	+0° 1′	+0° 3′	+1° 40′	+1° 39′	+1° 18′	1° 15′	25	..	.....
7	+0° 0′	0° 0′	+0° 56′	+0° 56′	+0° 22′	0° 22′	61	43	Bausch & Lomb, Blood cleared.
8	—0° 1′	—0° 1′	+0° 30′	+0° 31′	+0° 18′	0° 19′	39	40	Bausch & Lomb.
9	—0° 1′	—0° 1′	+0° 30′	+0° 31′	+0° 28′	0° 29′	6	0	Merck.
10	—0° 2′	0° 0′	+1° 0′	+1° 2′	+1° 3′	1° 3′	0	0	Merck.
11	—0° 2′	0° 0′	+1° 0′	+1° 2′	+0° 39′	0° 39′	38	..	Bausch & Lomb.
12	—0° 4′	0° 0′	+1° 4′	+1° 8′	+1° 7′	1° 7′	1	..	Merck
13	—0° 4′	0° 0′	+1° 4′	+1° 8′	+0° 39′	0° 39′	43	..	Bausch & Lomb.
			Aqueous glucose sol.		Aqueous glucose sol. cleared				
14	....	....	+0° 48′	.....	+0° 20′	.....	58.3	48.6	Bausch & Lomb.
15	....	....	+1° 0′	.....	+0° 25′	.....	58.3	..	.....
16	....	....	+1° 0′	.....	+0° 25′	.....	58.3	..	.....
17	....	....	+1° 1′	.....	+0° 26′	.....	58.7	..	.....
18	....	....	+1° 1′	.....	+1° 6′	.....	0	0	Merck.
			Diabetic urine		Diabetic urine cleared				
20	....	....	+6° 35′	.....	+5° 55′	.....	..	..	Bausch & Lomb.
21	....	....	+6° 35′	.....	+6° 38′	.....	..	..	Merck.

* Clearing always carried out with blood charcoal, 1 part to 10, in presence of 5 per cent. HCl.

wise pass unobserved. In this series, for special reasons, the urines were rendered only 2 per cent. acid, instead of 5 per cent. as according to Bang and Bohmannsson, and the weight of charcoal employed was smaller than these authors recommend. The glucose losses varied from a minimum of 2 per cent. to a maximum of 25 per cent., with an average of 15 per cent. The losses were not constant even in the same urines, but varied widely with the weight of charcoal used, the amount of shaking and the duration of contact. In Experiments 5 and 6, which represent two consecutive determinations on the same specimen of urine, the losses were 20 and 25 per cent. respectively. Occasionally two consecutive determinations gave the same figures, but not consistently.

Inasmuch as the statements of Bang and Bohmannsson are for the most part based on experiment with aqueous glucose solutions made 5 per cent. acid with hydrochloric acid, controls with such solutions were next made in exact accordance with the directions of these authors. In this series even higher percentage losses were encountered. Three determinations showed an average loss of 58 per cent. by polariscopic reading, and 48.6 per cent. by titration. Since the optical activity of the sugar solutions always fell more than their reducing power, we suspected the presence in the charcoal of an impurity that was either levorotatory, or of such a nature that it combined with the sugar to give reducing compounds having lower specific rotations than glucose. The possibility that the fall in rotation was merely a multirotational effect was eliminated by suitable precautions.

Since by extracting our charcoal with 5 per cent. aqueous hydrochloric acid we were unable to observe any optically active substance in the acid filtrate, and since charcoal so treated still exerted the same effect on glucose solutions as did the untreated charcoal, it seemed probable that we had to do with a substance in the blood charcoal that in some way combined with or metamorphosed the glucose.

With these considerations in view the experiments were continued with charcoal obtained from other sources. The original article was obtained from Bausch and Lomb; it was finely powdered and labeled "Charcoal from Blood." We made a second series of observations with Merck's "Blood Charcoal." This article had after pulverization a much coarser grain than the former. When used in the same proportion, the clearing was much less perfect than with the Bausch and Lomb product. Working with Merck's blood charcoal, however, we were able to confirm the findings of Bang and Bohmannsson and could demonstrate no significant loss of glucose from urinous or aqueous solutions, when these were cleared in the presence of a 5 per cent. hydrochloric acid.

Although it is naturally expected that pure chemicals be employed in the carrying out of any quantitative procedure, it is impossible in this connection to know beforehand, except by control experimentation, whether or not a given charcoal from blood will behave in the manner described by Bang and Bohmannsson, or otherwise, as in the present instance. Bang and Bohmannsson make no special mention as to what sort of *Blutkohle* shall be used. Different samples, however, acting in divergently opposite ways, make it necessary that, before any conclusions be drawn from measurements made by the Bang and Bohmannsson method, the individual sample of charcoal which is used shall be thoroughly tested in control experiments.

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