XXXIX. THE ABSENCE OF ABNORMAL FORMS OF GLUCOSE FROM BLOOD DIALYSATES.

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COMPARISONS of the optical activity and copper-reduction values of blood filtrates and dialysates have led certain observers to conclude that these aqueous solutions contain γ -glucose or other labile form of glucose [Winter and Smith, 1923; Lundsgaard and Holbøll, 1924, 1925; and in particular, Thomas, 1931]. Misapprehension of the behaviour of γ -glucose had led these workers to attribute to it the undoubted fact that the optical activities of blood dialysates are lower than the theoretical values deduced from the copper-reducing powers of the solutions.

Although numerous comparatively stable derivatives of γ -glucose (glucofuranose) are known, the free compound is unknown, and in aqueous solution instantly reverts to the ordinary equilibrated mixture of the stable α and β forms, showing $[\alpha]_D + 52.5^\circ$. (It is possible that a certain amount of γ -sugar may always be present in the equilibrium mixture but this would not then constitute an abnormality.)

To what then, can we attribute the discrepancies between reducing power and optical activity? Diminution of the rotatory power of glucose solutions has been shown to be caused by the presence of amino-acids and urea [Waterman, 1917, 1, 2, 1918; Euler *et al.*, 1926; Denis and Hume, 1924; Hynd, 1926, *etc.*]. While the concentrations within the blood of these substances are admittedly low, it should not be overlooked that their influence may have a slight effect in causing the differences noted.

Visscher [1926] was the first to attribute the phenomenon to the laevo-rotatory effect of salts of *d*-lactic acid, already shown by von Noorden [1912] to be formed from glucose by the glycolytic action of blood. In support of this view he called attention to the fact that the optical activity of blood filtrates altered with change in p_H . A similar effect was observed by Anderson and Carruthers [1926]. Wright, Herr and Paul [1928] succeeded in isolating zinc *d*-lactate from glycolysed blood, thus providing strong evidence in support of Visscher's theory.

In the recent paper by Thomas [1931] the work on *d*-lactic acid appears to have been disregarded, for the γ -glucose theory is again put forward. It was therefore considered expedient to endeavour to correlate the observed optical

activities of blood dialysates with the theoretical values calculated from both the sugar and the lactic acid contents. To this end fresh blood was obtained from cats, rabbits and sheep, some normal, others with high blood-sugar levels, induced by decerebration or by adrenaline injection, whereby it was possible to obtain dialysates with widely varying concentrations of glucose and of lactic acid.

The results of the experiments showed that the ideas of Visscher could be fully substantiated, as the amounts of lactic acid found in the dialysates were sufficient to account for the rotation discrepancies to well within the bounds of observational error. The polarimeter employed permitted the use of a 4 dm. tube and an accurate reading of 0.035° could be made. While errors of observation below 0.10° are unavoidably high, it will be seen that, in these experiments where the readings were greater than 0.035° , the mean error between observed and calculated activities was 0.020° .

EXPERIMENTAL.

Determination of specific rotation of sodium d-lactate.

Purdie and Walker [1893] quote $[\alpha]_D - 12.15^\circ$ for $c = 0.075 N$.

Concentration of sodium <i>d</i> -lactate %	α^{16°	$[\alpha]_D^{16^\circ}$
0.8752	-0.415°	-12.86°
0.3501	-0.208	-14.85
0.1403	-0.087	-15.50

For the following calculations sodium *d*-lactate was assumed to display the approximate $[\alpha]_D^{16^\circ} = -16.0^\circ$.

Dialysis of the blood and examination of the dialysates.

30 cc. of freshly drawn blood were dialysed in 10 portions of 3 cc. each into equal volumes of normal saline at 16° . After 30 minutes the clear dialysate was examined polarimetrically in a 4 dm. tube at 16° , the zero being determined immediately afterwards, with the same tube filled with distilled water. A portion of the dialysate was employed for the sugar estimation by a modified Shaffer-Hartman method, and the remainder, after removal of the sugar by the copper-lime precipitation method, was employed in the determination of the lactic acid by the procedure of Friedemann, Cotonio and Shaffer [1927].

Sources of blood employed.

Sheep (I to V) all normal.

Rabbits (VI to X). Normal (XI). Injected adrenaline, then amytal. Blood-sugar 334 mg./100 cc.

Cats (XII to XIV). Decerebrated (XV). Decerebrated and eviscerated.

In the experiments of Table I the amounts of sodium *d*-lactate were determined as lactic acid.

Table I. *Analytical data of dialysis experiments.*

Experiment	Sugar mg./100 cc.	Sodium <i>d</i> -lactate mg./100 cc.	Observed α °	α calculated from sugar °
I (Sheep)	18.4	15.4	+0.00	+0.039
II "	14.8	12.9	+0.035	0.031
III "	16.0	6.9	+0.00	0.034
IV "	16.8	nil	+0.035	0.035
V "	20.0	23.7	+0.028	0.042
VI (Rabbit)	38.4	87.3	+0.070	0.080
VII "	37.6	46.9	+0.070	0.079
VIII "	28.0	51.7	+0.035	0.059
IX "	28.0	41.8	+0.035	0.059
X "	24.8	70.2	+0.00	0.052
XI "	116.0	56.1	+0.208	0.254
XII (Cat)	164.0	38.0	+0.311	0.350
XIII "	78.4	51.1	+0.105	0.187
XIV "	88.8	103.4	+0.120	0.165
XV* "	47.2	117.5	-0.035	0.099

* The dialysis in this experiment was imperfect, as the dialysate had a faint pink tinge.

The values obtained from the sheep blood were too low to admit of polarimetric correlation; they are included however to demonstrate the presence of lactic acid in these dialysates.

Table II contains the polarimetric figures derived from those experiments in which the optical activity was greater than the minimum of discrimination (0.035°).

Table II.

Experiment	α_G is rotation calculated for % glucose found.			Observed α °
	α_G °	α_L °	$\alpha_G + \alpha_L$ °	
XII	+0.346	-0.015	+0.331	+0.311
XI	+0.245	-0.022	+0.223	+0.208
XIV	+0.187	-0.039	+0.148	+0.105
XIII	+0.167	-0.019	+0.148	+0.120
XV*	+0.099	-0.045	+0.054	-0.035
IX	+0.059	-0.015	+0.044	+0.035
X	+0.052	-0.027	+0.025	+0.00
IV	+0.035	0.00	+0.035	+0.035

It will be seen that in all the observations (excepting experiment XV where dialysis was imperfect), a mean difference is disclosed of only 0.020° between the calculated and the observed optical activities.

CONCLUSION.

It is concluded that in all the specimens of blood examined, the apparent low optical activities of the dialysates may be accounted for by the presence of the laevorotatory sodium *d*-lactate, without assuming the presence of any γ -glucose.

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