published microbiological methods of assay. *K. brevis* was found to be satisfactory for the assay of pantothenic acid and inositol, *Saccharomyces cerevisiae* var. *ellipsoideus* for pantothenic acid, *S. macedoniensis* for aneurin, *Candida guiller-*

mondia for biotin and *Schizosaccharomyces pombe* for inositol.

3. *Kloeckera brevis* was used for the assay of pyridoxin, but the results were frequently at variance with those given by *Neurospora sitophila*.

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The Sedimentation Constant of Insulin

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In view of other evidence, to be published elsewhere by one of us (H.G.) on the molecular size and stability of insulin, we undertook a re-examination of the sedimentation constant over a range of pH and at different concentrations. We are unable to agree with Sjögren & Svedberg (1931) and Miller & Andersson (1942) about the value of the sedimentation constant or the range of stability. Sjögren & Svedberg found that a fall in the sedimentation constant occurs above pH 7 and below pH 4.5. Miller & Andersson confirmed a fall between pH 7.5 and 8.5 and also concluded that at pH 8.5 the insulin is becoming heterogeneous. Miller & Andersson obtained a sedimentation constant of 3.55×10^{-13} at pH 7 which agreed well with Sjögren & Svedberg's uncorrected value of 3.47×10^{-13} . Both these workers used recrystallized insulin. Miller & Andersson worked at a concentration of 1 % insulin.

EXPERIMENTAL

Sedimentation was observed in a Svedberg oil-turbine ultracentrifuge by the method of Philpot (1938); the speed was 1000–1020 rev./sec. In view of the difference of our results from those of previous workers we should mention the precautions that have been taken to make our estimates of the sedimentation constant accurate: the constants of the optical system were carefully and directly measured (by A. G. Ogston and J. P. Johnston); the vibrating reed frequency-meter had been calibrated against a crystal oscillator (with the kind collaboration of Dr R. P. Martin of the Clarendon Laboratory) and had been shown to give stable readings over a period of 2 years; the reading of the rotor temperature had been checked at 300 rev./sec. (Ogston, 1943) and found correct; in addition we found that the thermocouple reads correctly the temperatures encountered at 1000 rev./sec., but we have not tested whether these temperatures actually correspond with those of the rotor.

The materials, concentrations and buffers used, together with the sedimentation results are shown in Table 1. The solutions were made up either by solution into the buffer or by dialysis against a large volume of buffer. The final values of pH were measured with a glass electrode and Cambridge pH-meter.

but neither of these differs significantly from it (no. 5, $P=0.7$; no. 8, $P=0.1$): it is likely, however, that the lower value at 1.24% protein concentration does represent a normal decrease of the sedimentation constant with rise of concentration.

The value of S_{20} at pH 3.4 is significantly lower than the mean ($0.02 > P > 0.01$).

Table 1. *Sedimentation constant of insulin*

	Specimen of insulin	Concentration (%)	pH	Buffer				$S_{20}(\text{corr.}) \times 10^{13}$
				Citric acid (M)	NaCl (M)	Na_2HPO_4 (M)	KH_2PO_4 (M)	
(1)	Squibb crystalline	0.4	3.4	0.055	0.1	0.045	—	3.09 ± 0.15
(2)	Squibb crystalline	0.15	6.7	—	0.1	0.005	0.005	3.39 ± 0.09
(3)	Squibb crystalline, electro dialyzed	0.25	6.8	—	0.1	0.005	0.005	3.33 ± 0.11
(4)	Squibb crystalline	0.83	7.1	—	0.1	0.0095	0.0005	3.30 ± 0.06
(5)	Boots crystalline	1.0	7.3	—	0.1	0.0095	0.0005	3.37 ± 0.08
(6)	Boots crystalline	0.25	7.7	—	0.1	0.0095	0.0005	3.43 ± 0.12
(7)	Boots crystalline	0.10	7.8	—	0.1	0.0095	0.0005	3.30 ± 0.08
(8)	Squibb crystalline	1.24	8.7	—	0.1	0.091	—	3.22 ± 0.04
(9)	Squibb crystalline	0.34	9.05	—	0.1	0.10	—	3.35 ± 0.05
(10)	Boots amorphous* (Blatherwick <i>et al.</i> 1927)	1.0	7.15	—	0.1	0.008	0.002	3.35 ± 5.12
(11)	Boots crystalline	0.31	8.6	Na veronal, 0.015 M; HCl, 0.044 M				3.42
(12)	Boots crystalline	0.49	8.6	NaOH, 0.068 M; NaCl, 0.231 M				3.32
(13)	Boots crystalline	0.43	9.6	Na veronal, 0.02 M; NaCl, 0.40 M				3.30

Weighted mean of 2, 3, 4, 6, 7, 9 = 3.34 ± 0.03 .

* Contained two components, the slower being 80% of the whole.

The standard deviation of each mean value of the sedimentation constant was estimated from the values for intervals in each run and is given against the mean value in the table. The values of the standard deviations obtained are apt to be too high, since an error in measuring the mean position of the boundary in a single photograph causes the values of S_{20} obtained from the previous and successive intervals to err in opposite directions, without affecting their mean. The values of S_{20} are therefore more accurate than they appear. However, these estimated standard deviations have been used in estimating the significance of differences.

At the end of Table 1 are given three values for the sedimentation constant of insulin obtained by Dr E. R. Holiday with the Oxford ultracentrifuge. These values are quoted with his permission.

DISCUSSION

The weighted mean value of our measurements (runs 2, 3, 4, 6, 7, 9) between pH 6.7 and 9.05 is 3.34 ± 0.03 (standard deviation of mean). None of the values contributing to this mean differs significantly ($P > 0.05$) from any of the others or from the mean. We have omitted from this mean the two values obtained at concentrations of 1% or over,

Between pH 6.7 and 9.05 and even up to pH 9.6, there is no evidence from our results that there is any change in the sedimentation constant of crystalline insulin; nor do we find in our diagrams any sign of abnormal spreading of the boundary, though increased spreading is clearly evident at pH 3.4. There is no significant change of sedimentation constant with concentration below 1%.

It is difficult to understand why our results should differ from those obtained by previous investigators. The two chief sources of error in determining sedimentation constants are the measurement of the speed of rotation which contributes 0.2% error/1 rev./sec. of error at 1000 rev./sec., and the measurement of the temperature of the cell contents which contributes an error of 2.1%/degree of error at 25°. Thus, in order to account for the difference of 6.3% between our value of 3.34 and Miller & Andersson's of 3.55 for S_{20} , an error of about 30 rev./sec. or of 2.7° is required. Assuming that Miller & Andersson's result is correct, our measurement of the speed must have been 30 rev./sec. too high or of the temperature 2.7° too high. We do not believe that our measurement of the average speed is wrong by more than 2 rev./sec. and we find it difficult to believe that our estimates of the rotor temperature can be wrong by more than a few

tenths of a degree. Our confidence in our value of the sedimentation constant is increased by the agreement between three different samples, one of them amorphous and containing a second component.

SUMMARY

1. The sedimentation constant of insulin has been determined over a range of concentration of 0.1 to 1.24 % and of pH from 3.4 to 9.05. The sedimentation constant was found not to vary significantly between pH 6.7 and 9.05 at concentrations of

less than 1 %. Its mean value was $3.34 \pm 0.03 \times 10^{-13}$. At pH 3.4 the sedimentation constant was significantly lower and the insulin markedly polydisperse.

2. These results differ from those of previous workers with respect to the value of S_{20} and to the stability of insulin at pH greater than 7.

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