CXC. THE BLOOD-SUGAR LEVEL IN VITAMIN B₁ DEFICIENCY.

By MURIEL EMMA BELL¹.

*From the Department of Physiology and Biochemistry, University College,
London.*

(Received September 1st, 1931.)

THERE is still some uncertainty about the reducing value of blood in vitamin B deficiency. The recent contributions on this subject include that of Drummond and Marrian [1926], who found a rise in the blood-sugar of rats fed on a diet completely deficient in B-vitamins, and that of Sure and Smith [1929], who seemed to have provided an explanation when they reported that the rise was due to non-sugar reducing substances in the blood of vitamin B₁-deficient rats. Drummond and Marrian had used the original Hagedorn-Jensen method; Sure and Smith applied Folin's ferricyanide reduction method [1928] to the filtrates obtained by using the Folin-Wu tungstate precipitation [1919] and the Somogyi zinc hydroxide precipitation [1928]. In view of further recent work on the nature of the reducing substances of blood, it seemed desirable to re-investigate the problem, and the original idea was to ascertain what non-sugar reducing substance was responsible for the apparent rise in sugar.

As a preliminary to employing the methods at present regarded as orthodox [Cori, 1931] for arriving at "true sugar" and non-sugar reducing values, series of human bloods and of pigeons' bloods were analysed to compare the Somogyi and the Hagedorn-Jensen zinc filtrates. The Hagedorn-Jensen ferricyanide reduction was used throughout; the filtrates used were the Folin-Wu for total reduction, the Somogyi zinc filtrate, and, in pigeons, the unaltered filtrate of Herbert and Bourne [1930, 1] for "true sugar" values.

It has been shown that for normal and pathological human bloods [Herbert and Bourne, 1930, 2], the non-sugar reducing fraction runs parallel with the cell volume, as the greater part of this fraction is contained in the corpuscles. Accordingly, the cell volume was determined by half-an-hour's centrifuging in haematocrit tubes at 1500 revolutions. Sure and Smith's results seemed to indicate that there would be a very large increase in the cell volume or that there must be some entirely new reducing substance present in bloods from vitamin B₁-deficient animals.

In the following series, the bloods both of pigeons and of rats during normal and vitamin B₁-deficient periods, have been studied. Pigeons were studied in the first instance, partly because they could better spare the blood needed for

¹ William Gibson Research Scholar.

repeated bleedings and for duplicate estimations, partly because they have a higher figure than rats both for sugar and for non-sugar reducing substances, as shown by Gulland and Peters [1930]; hence the risk of experimental error of any magnitude, with the micro-methods which must be used, would be lessened.

EXPERIMENTAL.

Comparison of Hagedorn-Jensen with Somogyi zinc filtrates from human-blood.

It may seem unnecessary to produce further evidence that the Hagedorn-Jensen filtrate does not give absolutely true sugar values when several workers have already confirmed Somogyi's results [Herbert and Bourne, 1930, 1; Ege and Roche, 1930]; but as the Hagedorn-Jensen method of precipitation is still widely used, and as, for example, Gulland and Peters [1930] state that "zinc filtrates" (unqualified) "do not contain aliphatic —SS— and —SH— compounds," and that "zinc filtrates are the most reliable for estimating reducing substances in avian blood," it seemed advisable to go over this ground once more in order to see whether there were any significant differences between the two methods of zinc hydroxide precipitation. Somogyi claims that adjusting the reaction to neutrality or slight alkalinity not only precipitates the proteins completely, but also removes the non-sugar reducing substances.

In a recent communication, Kramer and Steiner [1931] have raised an objection to the use of cotton-wool plugs for filtering the coagulated proteins on the ground that they retained some of the sugar. Comparing this filtrate with that of Somogyi they obtained values of 20–30 mg. higher for human blood with the former than with the latter; Somogyi's [1930] comparison between these two methods similarly gave differences of 20–30 mg. On the other hand, Herbert and Bourne found values which were only 1–10 mg. higher; Ege and Roche similarly obtained only a 5–15 mg. difference. Ferricyanide reduction was used in all these instances. The discrepancies were explained by Kramer and Steiner by the inadequate washing of the cotton-wool plugs. In a private communication Herbert and Bourne state that they used, not cotton-wool, but 4 cm. filter-papers, and that a third washing did not alter their result. In order to clear up this point, the following procedure was adopted, using a micro-technique and ferricyanide reduction.

1. Somogyi's No. 1 procedure, *i.e.* using 1.8 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.1 *N* NaOH. The solutions were adjusted and precipitation effected as specified by Somogyi.

2. It will be observed that the actual amount of zinc sulphate added is four-fifths of that used in the Hagedorn-Jensen filtrate. (It may be remarked in passing that Hagedorn and Jensen do not state specifically in their original paper that it is the $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ which is to be used, but it is apparently the common practice to use the salt containing water of crystallisation.) Therefore, in order to make the two methods comparable in all respects except in the amount of zinc sulphate added, the Hagedorn-Jensen filtrate was prepared

according to the following directions. To 0.2 cc. blood, laked in 5.5 cc. water add 1.25 cc. of 1.8 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.0 cc. of 0.1 *N* NaOH. Place in boiling water-bath for 3 minutes. Filter through filter-paper (starch-free). Take 5 cc. of the filtrate. The procedure was the same, therefore, for the Hagedorn-Jensen as for the Somogyi technique, except that the amount of zinc sulphate was 1.25 times greater in the former than in the latter, and that there had been no application of heat in the latter. The following points were attended to: the amount of fluid in the boiling-tubes was made up to 14 cc.; the tubes were covered during and after reduction to minimise re-oxidation; the ferricyanide was twice recrystallised from a "recrystallised" sample. Care was taken to see that the blood was adequately mixed, to ensure even distribution of the corpuscles.

The figures obtained for human blood are given in Table I. It will be seen that the differences range from 5 to 15 mg. with an average of 8, which compares very closely with those recorded by Herbert and Bourne and by Ege and Roche.

Table I. *Comparison between Somogyi and Hagedorn-Jensen filtrates.*
Human blood.

Sample	Sugar value (mg./100 cc.)		Difference
	Somogyi filtrate	Hagedorn-Jensen filtrate	
1	90	105	15
2	116	123	7
3	105	112	7
4	102	109	7
5	90	99	9
6	64	69	5
7	73	86	13
8	88	93	5
9	70	80	10
10	80	86	6
			Average 8

The cotton-wool plugs accordingly do not seem to explain the discrepancy, as suggested by Kramer and Steiner. The latter state that they used 0.30 to 0.35 cc. *N*/10 NaOH to neutralise their filtrate. As it seemed possible that they might perhaps have made their zinc sulphate solution to contain 0.45 % of ZnSO_4 instead of 0.45 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, a few human bloods were tested using the additional zinc sulphate, neutralising and not neutralising the filtrate, and again taking an aliquot part of the filtrate. The effect of the extra zinc sulphate was to increase the difference in reduction between the Somogyi and the second filtrate only if the latter were neutralised. Apparently, not neutralising the filtrate diminished the efficacy of the ferricyanide reagent, with the result that the figures were much the same as the ordinary Hagedorn-Jensen figures.

Herbert and Bourne have shown that the p_{H} of the Hagedorn-Jensen filtrate is 6.8, while that of the Somogyi filtrate is 7.4. In this investigation, the p_{H} was similarly determined on a few samples, using the method of Dale and Evans [1920]. The average values thus obtained were 6.85 and 7.2 re-

spectively. It would seem, therefore, that the critical p_H is the neutral point, as Somogyi claims.

Reducing values for pigeons' blood.

Whereas the evidence put forward by several different workers is that the two zinc filtrates give slightly different results for human blood, the difference is more marked when they are applied to avian blood, with its greater content of non-sugar reducing substances. It was stated by Gulland and Peters that 70 mg. in terms of glucose was the average for the non-sugar reducing substances of pigeons' blood. They, however, used the Hagedorn-Jensen filtrate to give total reduction, and blood which has been allowed to undergo slow spontaneous glycolysis to obtain their residual reduction. The Hagedorn-Jensen filtrate, as Table II shows, does not allow through more than a part of

Table II. *Comparison between Hagedorn-Jensen¹ and Somogyi filtrates. Pigeons' blood.*

No.	(mg./100 cc.)			Difference
	"Total sugar" (Folin-Wu filtrate)	Somogyi filtrate	Hagedorn-Jensen filtrate	
1	256	192	208	16
2	256	198	230	32
3	227	176	207	31
4	254	198	224	26
5	315	259	291	32
6	334	279	300	21
7	230	163	190	27
8	227	176	207	31

the non-sugars, and it is possible that blood after slow glycolysis has not lost all its glucose. Moreover, in the absence of glucose, as Herbert, Bourne and Groen [1930] have shown, glutathione has a rather higher glucose equivalent. It is suggested, therefore, that the figure of 70 mg. was obtained by a compensation of errors.

Blood was obtained by puncturing the wing vein, the bird being kept quiet by putting its head under the opposite wing. The blood was rapidly mixed with a minimal quantity of oxalate. 0.1 cc. blood was used for an estimation in each case.

Normal birds were fed with poultry feed. The deficient diet offered was polished rice and Kon and Drummond's diet [1927]. When the birds refused to eat the deficient diet, they were forcibly fed with the latter. About 15 g. were placed in the crop each day; this was insufficient to maintain their weights, but ensured that they were not suffering from starvation.

It will be seen (Tables III and IV) that the average non-sugar reducing value for pigeons' blood by these methods is about 60 mg. in terms of glucose. The cell volume averages 49 %. For human blood, the corresponding figures are 20-30 mg. and 41 % respectively [Herbert and Bourne, 1930, 2]. The

¹ Using an aliquot part of the filtrate.

Table III. *Normal pigeons fasting 24 hours.*

Bird	Date	Reduction value expressed as mg. glucose/100 cc. blood				Cell volume %
		"Total sugar" Folin-Wu filtrate	"True sugar"		Non-sugar reducing substances	
			Somogyi filtrate	Isotonic filtrate		
"Squeakers"						
734	8. vii. 31	—	130	—	—	—
734	17. vii. 31	206	133	130	73	51
736	8. vii. 31	243	182	—	61	—
736	17. vii. 31	264	202	206	62	—
742	8. vii. 31	206	150	—	56	46
742	17. vii. 31	184	130	133	54	—
478	8. vii. 31	203	136	139	67	51
478	17. vii. 31	224	154	—	70	52
731	8. vii. 31	205	150	150	55	46
731	17. vii. 31	221	154	—	67	—
	Average	217	152	—	63	49
Adult birds						
p.s.r.	19. v. 31	249	181	178	68	49
1707	19. v. 31	243	179	—	64	—
9754	19. v. 31	245	179	181	66	50
1478	19. v. 31	252	194	—	58	45
p.p.r.	20. v. 31	238	179	174	59	50
9738	20. v. 31	229	167	167	62	51
9731	22. v. 31	254	198	198	56	—
9743	22. v. 31	237	176	176	61	47
	Average	243	182	—	62	49

Table IV. *Normal pigeons feeding.*

Bird	Date	Reduction value expressed as mg. glucose/100 cc. blood				Cell volume %
		"Total sugar" Folin-Wu filtrate	"True sugar"		Non-sugar reducing substances	
			Somogyi filtrate	Isotonic filtrate		
"Squeakers"						
736	2. vii. 31	246	195	—	51	—
734	2. vii. 31	242	174	—	68	55
731	2. vii. 31	253	184	184	69	47
742	2. vii. 31	235	165	165	70	49
478	2. vii. 31	216	162	—	54	51
	Average	238	176	—	62	50
Adult birds						
9742	25. iv. 31	249	195	192	54	43
375	27. iii. 31	283	229	—	54	—
375	25. iv. 31	293	228	222	65	54
9736	31. iii. 31	246	187	187	59	—
p.p.	31. iii. 31	278	—	—	—	—
9735	7. iv. 31	258	206	—	52	—
1478	7. iv. 31	248	203	200	45	—
m.f.	8. iv. 31	268	208	202	60	—
f.w.	8. iv. 31	246	187	—	59	—
9731	8. iv. 31	253	195	—	58	46
9743	8. iv. 31	264	197	200	67	45
1707	9. iv. 31	254	206	—	48	—
9738	10. iv. 31	277	216	—	61	—
p.w.	17. iv. 31	—	212	213	—	—
p.s.r.	26. iii. 31	256	192	—	64	—
p.w.	26. iii. 31	256	198	—	58	47
	Average	262	204	—	58	47

non-sugar reducing content is consequently higher per unit volume of corpuscles in birds' blood than in human blood.

The Hagedorn-Jensen filtrate (Table II) gives values 16–32 mg. higher than the Somogyi filtrate, but not as high as the Folin-Wu filtrate. When these filtrates were tested by the nitroprusside reaction, the Folin-Wu gave a markedly positive colour, the isotonic and the Somogyi were negative, while the Hagedorn-Jensen gave a colour about half as intense as the Folin-Wu. This lends support to the view that the substance mainly concerned in producing non-sugar reduction is glutathione or other sulphhydryl-containing substances.

The "true sugar" averages 152 mg. for young birds fasting 24 hours, 182 mg. for adult birds fasting 24 hours; 176 mg. for young birds feeding, 204 mg. for adult birds feeding. The range of figures is a wide one, namely from 130 mg. to 198 mg. for fasting birds, from 162 mg. to 230 mg. for feeding birds. This wide range is probably to be explained by the varying degrees of excitement that the manipulations produce. From the small group of young birds studied, the impression was gathered that the smaller birds had lower amounts of circulating glucose, perhaps connected with their greater intensity of metabolism and more rapid utilisation of glycogen stores. Gulland and Peters deduced from their average of 135 ± 15 mg. glucose that "the true sugar value of birds' blood is not so widely divergent from that of mammals as the Hagedorn-Jensen estimations seem to show." With the methods used here, the figures are higher, in spite of the fact that their method of collecting the sample—by guillotining the bird—is likely to lead to a rise in blood-sugar. One is of the opinion, therefore, that their figures for true sugar are definitely low.

The two methods for true sugar were undertaken, firstly in order to see what effect the extra amount of uric acid contained in birds' blood had on the unlaked filtrate of Herbert and Bourne. Their filtrate is practically identical with that simultaneously proposed by Folin [1930]. It is unlikely that the uric acid would be confined to the corpuscles, and yet the figures show rather surprising agreement between the unlaked and the Somogyi filtrate. The figures given by Folin and Denis [1913] for uric acid in birds' blood are 4.8 mg.; those given by Scheunert and Pelchrzim [1923] are about 2 mg. The reducing value of uric acid, using ferricyanide reduction, is 53 % that of glucose [Hagedorn and Jensen, 1923, 2; Holden, 1926], so that the maximum difference due to uric acid would be 2.4 mg. in terms of glucose, even if it all diffused out of the corpuscle in the time allowed. The second reason for using the unlaked filtrate was to see whether the alleged rise in non-sugar reducing substances in vitamin B₁-deficiency were due to a constituent of the cells or of the plasma; if it were a non-diffusible substance present in the cells, then the isotonic filtrate would show a smaller value than the Somogyi filtrate. It was found, as later results indicate, that the two filtrates gave very similar results during the stage of vitamin B₁-deficiency in pigeons.

Table V. *Vitamin B₁-deficient pigeons during convulsions.*

Bird	Date	Reduction value expressed as mg. glucose/100 cc. blood				Cell volume %
		"Total sugar" Folin-Wu filtrate	"True sugar"		Non-sugar reducing substances	
			Somogyi filtrate	Isotonic filtrate		
Adult birds						
p.s.r.	13. iv. 31	507	461	—	46	37
	14. iv. 31	392	336	336	66	35
9754	18. iv. 31	264	197	198	66	43
	19. iv. 31	314	—	258	56	45
	20. iv. 31	382	312	312	70	44
	21. iv. 31	333	—	—	—	—
p.p.	18. iv. 31	340	282	288	58	39
	19. iv. 31	370	314	325	56	37
	20. iv. 31	298	—	261	37	35
9738	22. iv. 31	298	235	228	63	—
f.w.	22. iv. 31	320	275	273	45	38
	23. iv. 31	480	438	419	42	—
	24. iv. 31	—	509	—	—	—
m.f.	23. iv. 31	344	296	296	48	—
	24. iv. 31	299	256	267	43	—
1478	27. iv. 31	406	330	—	76	—
	28. iv. 31	296	232	—	64	—
	29. iv. 31	283	243	245	40	35
9743	28. iv. 31	296	248	251	48	40
	29. iv. 31	259	221	230	38	37
1707	1. v. 31	—	237	243	—	40
	2. v. 31	279	229	235	50	—
9731	8. v. 31	242	197	—	45	32
p.w.	15. v. 31	374	317	306	57	44
9736	23. v. 31	338	253	—	85	48
p.s.	11. vi. 31	262	219	222	43	40
	12. vi. 31	355	296	—	59	—
	13. vi. 31	—	283	—	—	—
	14. vi. 31	315	232	—	83	42
9731	15. vi. 31	—	295	—	—	—
9734	15. vi. 31	340	275	—	65	—
9742	17. vi. 31	229	155	156	74	51
	17. vi. 31	248	190	—	58	—
	18. vi. 31	289	—	228	61	53
	18. vi. 31	412	350	—	62	52
	19. vi. 31	459	398	396	61	52
p.w.	18. vi. 31	515	434	440	75	41
	18. vi. 31	506	459	—	47	—
	19. vi. 31	367	314	—	53	45
9743	19. vi. 31	—	248	251	—	39
p.p.	20. vi. 31	278	—	224	54	48
	20. vi. 31	314	267	—	47	47
1478	20. vi. 31	294	249	—	45	44
9736	21. vi. 31	—	262	—	—	—
	Average	340	290	—	53	41
"Squeakers"						
478	30. vii. 31	334	—	279	55	42
	31. vii. 31	302	253	—	49	43
	1. viii. 31	302	256	—	46	40
	2. viii. 31	—	253	—	—	40
	3. viii. 31	—	267	—	—	—
731	31. viii. 31	534	499	—	—	44
	1. viii. 31	397	336	—	61	—
742	5. viii. 31	251	195	—	56	—
	6. viii. 31	235	165	—	70	60
	6. viii. 31	236	—	151	85	65
734	6. viii. 31	211	170	—	41	—
	7. viii. 31	—	224	—	—	—
	Average	311	272	—	59	49

Blood-sugar changes in pigeons during vitamin B₁-deficiency.

The changes that occurred in "true sugar" during vitamin B₁-deficiency can be seen in Tables V, VI, VII and Fig. 1. During the stage of convulsions, the "true sugar" went up—parallel with the "total sugar"—to an average value of 290 mg. for adult birds and 272 mg. for the group of young birds.

Table VI. "True sugar" in blood of pigeons during pre-convulsive stage.

Bird	Date	"True sugar" (Somogyi filtrate) mg./100 cc.	Bird	Date	"True sugar" (Somogyi filtrate) mg./100 cc.
734	4. viii. 31	144	9735	12. v. 31	163
742	4. viii. 31	190	375	12. v. 31	190
734	31. vii. 31	154	p.w.	11. v. 31	182
731	31. vii. 31	224	9731	7. v. 31	218
742	31. vii. 31	176	p.p.r.	16. iv. 31	179
1478	16. vi. 31	173	9738	16. iv. 31	172

Table VII. "True sugar" in blood from heart of pigeons, dead from vitamin B₁ deficiency.

No. 742, at death, following convulsions	...	151 mg.
„ 9744, „ (from ascites)	...	312 mg.
Blood from heart after death:		
No. 478	...	90 mg.
„ 731	...	not less than 256 mg.
„ 9731	...	27 mg.
„ 9734	...	275 mg.
„ p.s.r.	...	128 mg.
„ 734	...	378 mg. (dead in position of head retraction)

Fig. 1. Graphs of increased reduction in blood of pigeons during convulsions.

Occasionally the "true sugar" reached a figure of 500 mg. Of the twenty birds used, most of which were studied through two periods of deficiency, there was no exception to the finding that a given individual bird will show an increase over its fasting level for sugar, and usually over its feeding level also. The response varied considerably with different individual birds.

The non-sugar reducing substances suffered no appreciable alteration. Occasional high values were attributed to experimental error. The average value was 53 mg., in terms of glucose, for one group of animals, a rather lower figure than the normal average. In this group, furthermore, the average cell volume diminished slightly. Some birds showed a progressive anaemia—and the non-sugar reducing substances followed the cell volume downwards. This is consistent with the finding that the greater part of the non-sugar reducing substances resides in the corpuscles. Occasionally, on the other hand, a bird which developed ascites showed an increase both in cell-volume and in non-sugar reducing substances. Bird 742 is an extreme example of this, where the cell volume at the time of death was 65 %, and the non-sugar value was 85 mg. This was, however, exceptional. The bird had lost water not only into its tissues, but also by the rapid respiration, presumably due to hydropericardium, which it had been experiencing for several hours before death.

Occasionally a bird which did not develop convulsions had a high "true sugar" level, *e.g.* bird 9744 died during manipulations for taking the blood sample. It was found to have ascites, with fluid in the pericardial cavity. Its "true sugar" was at the high level of 342 mg. Undoubtedly an asphyxial element would enter into the production of hyperglycaemia in this case. Only twice was it possible to get blood from the heart immediately after death from convulsions. No. 742 whose temperature was 26° at death had a "true blood-sugar" of 150 mg.; No. 9742 died half-an-hour after the last sample containing 390 mg. had been taken. Even among the birds which had been dead for several hours before their heart-blood was collected, there were three with figures of 256, 275, and 378 mg. respectively. The lower figures of 90 and 27 mg. may have resulted from glycolysis, but may also have been similar findings to those of Drummond and Marrian who frequently found low values at the time of death.

On the whole, in this series, the incidence of hyperglycaemia has been associated with the convulsive stage. It was seldom that any rise occurred in the pre-convulsive stage (Table VI). Extraordinarily high figures were reached in some cases where convulsions were present. A sub-lethal dose of cyanide was accordingly administered to two pigeons to ascertain the effect of convulsions produced in this way. The first developed typical convulsions and gave an increase in "true sugar" from 131 to 259 mg. The second, dosed on two occasions, did not become convulsed, and showed a diminution from 139 to 128 mg. and from 160 to 149 mg. The hyperglycaemia in the first pigeon would no doubt come under the heading of an asphyxial rise in sugar [Macleod, 1926].

An attempt to prove that the raised blood-sugar was due to an increased output of adrenaline was unsuccessful.

Sugar values for rats' blood.

Since vitamin B₁-deficient pigeons had shown no rise in non-sugar reducing substances but were definitely hyperglycaemic during the convulsive stage, rats were now used as the experimental animals.

Normal values were first established and were found to lie, as Sure and Smith stated, within the values of 17–34, with an average of 29 mg. per 100 cc. of blood, in terms of glucose. In the first estimations, a number of (feeding) stock rats were used; they were killed with chloroform, and blood was taken from the heart. A few (feeding) vitamin B-deficient rats were similarly used. For the great majority of the figures, however, blood was taken from the lateral vein of the tail of animals fasting 6 hours or longer. From 0.2 to 0.3 cc. blood was collected with a pipette into a minimal quantity of oxalate.

The vitamin B₁-deficient animals were fed on a diet consisting of rice starch 75 %, caseinogen 20 %, salt mixture 5 %. A supplementary ration of 1 drop of cod-liver oil, and of autoclaved marmite (to supply vitamin B₂) was fed to all the rats; the controls received an additional ration of Peters's extract (vitamin B₁). The animals fasted for at least 6 hours before the sample was taken.

Normal values.

(a) "Total sugar"—usually below 120 and above 80 mg., with an average of 109 mg.

(b) "True sugar"—usually below 90 and above 50 mg., with an average of 76 mg.

(c) Cell volume, average value 44 %.

(d) Non-sugar reducing substances, average 29 mg. in terms of glucose.

The vitamin B-deficient animals were classified into those that had convulsions, those that did not develop convulsions, and those that were moribund when the sample was taken (Table VIII). The average cell volume for these groups was 44, 46, and 44 respectively. No consistent alteration occurred in the cell volume. The non-sugar reducing substance averaged 32, 28 and 27 mg. respectively, and in no case was it over 35 mg. Care was taken, when it was suspected that the "real sugar" might be low, to increase the quantity of blood taken for the Somogyi estimation from 0.1 to 0.15 cc. or more if it could be obtained. Duggan and Scott [1926] and Kramer and Steiner [1931] have thrown doubt on the accuracy of estimations of amounts below 25 mg. If that is the case, then a Somogyi estimation, since it is done on five-eighths of the filtrate, must be over 40 mg. to be accurate.

While, contrary to Sure and Smith's finding, no increase was found in the non-sugar reducing value of the blood of vitamin B-deficient rats, on the other hand, in agreement with Sure and Smith, no increase in the "true sugar" was

Table VIII. *Vitamin B-deficient rats, fasting 6 hours.*

(Convulsions.)					
No.	Date	"Total sugar"	"True sugar"	Non-sugar reducing substances	Cell volume
124	9. vii. 31	71	41	30	44
49	9. vii. 31	123	91	32	43
50	9. vii. 31	63	28	35	—
121	9. vii. 31	96	68	28	—
941	12. vii. 31	—	83	—	—
48	14. vii. 31	111	77	34	—
119	18. vii. 31	101	72	29	45
125	20. vii. 31	112	79	33	—
Average		97	67	32	44
(No convulsions. Hunched.)					
43	29. vi. 31	75	53	22	—
40	2. vii. 31	98	75	23	—
122	2. vii. 31	117	100	17	42
117	11. vii. 31	143	118	25	44
126	5. viii. 31	91	74	17	—
128	7. viii. 31	110	78	32	48
125	7. viii. 31	103	74	29	48
126	7. viii. 31	99	64	35	48
43	7. viii. 31	112	83	29	42
46	7. viii. 31	100	70	30	—
50	10. viii. 31	96	—	—	45
49	10. viii. 31	120	96	24	41
129	12. viii. 31	90	64	26	—
133	12. viii. 31	86	54	32	47
132	12. viii. 31	83	54	29	46
131	12. viii. 31	96	72	24	—
130	12. viii. 31	102	68	34	—
132	19. viii. 31	82	56	26	—
51	20. viii. 31	101	72	29	—
39	18. vi. 31	106	74	32	46
40	18. vi. 31	102	70	32	45
Average		101	73	28	46
(Moribund.)					
52	29. vi. 31	120	90	30	42
42	2. viii. 31	130	101	29	—
123	10. vii. 31	67	38	29	—
122	10. vii. 31	—	7	—	—
118	10. vii. 31	40	15	25	46
119	20. vii. 31	25	3	22	—
Average		77	42	27	44

observed. Not even during the stage of convulsions did the rats show a hyperglycaemia. Then began a process of elimination to account for the discrepancy between the findings in this series and the finding by Drummond and Marrian—that a “hyperglycaemia occurs in rats during the greater part of the time that they are on a vitamin B-deficient diet.” Firstly, the Hagedorn-Jensen method was too little different from the Somogyi method to account for the discrepancy. Secondly, the diet given by Drummond and Marrian was deficient in B-complex; therefore a few rats deficient in vitamin B₂ only were used, to see whether this was the factor responsible, with the result that they also showed the same normal sugar values. Thirdly, as it was found that Drummond and Marrian had used blood taken from the heart immediately after rapidly

Table IX. "*True sugar*" values for rats' blood, showing effect of method by which blood is collected.

(Rats fasting overnight.) "True sugar" values (Somogyi filtrate).			
mg./100 cc. blood			
Normals No.	Blood from tail	Blood from heart, immediately after rapid death by dislocation of the neck	Rise produced by latter procedure
168	82	112	30
169	74	107	33
173	52	52	0
175	102	106	4
166	106	129	23
170	102	171	69
134	89	96	7
135	85	152	67
172	96	103	7
171	79	126	47
165	70	106	36
1	71	120	49
2	83	96	13
3	68	129	61
4	58	86	28
5	78	96	18
Vitamin B ₁ -deficient:			
132	56	79	28
125	79	118	39
51	62	79	17
133	48	82	34
130	40	49	9
49	81	139	58

killing the animals by dislocating the neck, a few vitamin B₁-deficient animals were treated in this way. Blood was first taken from the tail, the animals were rapidly killed and blood was taken immediately from the right side of the heart. These latter samples gave higher values than the tail-blood samples. In a series of normal rats treated in the same way, a similar rise was observed in the majority of the second samples. Sometimes there was no appreciable rise. The increase ranged from 0 to 69 mg. (Table IX). This means that it is possible to have something like a 100 % error if blood is collected by this method for sugar estimations. Apparently this fact has not been generally recognised hitherto. The rise occurs with extraordinary rapidity. The physiological explanation is either that of intense excitement before death, or direct stimulation of the spinal cord by the odontoid process of the second cervical vertebra as it penetrates the spinal cord; adrenaline secretion will occur and glycogen be mobilised. As evidence of adrenaline secretion, the blood clotted very rapidly.

No explanation, therefore, has been found for the definitely lower values for blood-sugar found by Drummond and Marrian to occur in the blood of normal rats as compared with vitamin B-deficient rats and at present it appears to be due to coincidence. It will be seen by reference to Table IX, that there is room for that coincidence to have occurred.

DISCUSSION.

These results seem to explain why a hyperglycaemia in pigeons has not always been found. Funk and Schönborn [1914] and several other workers found a rise, but Kon and Drummond [1927] reported no difference in the sugar levels of the two groups of animals which they studied.

It appears to depend upon the stage at which the blood-sample is taken, the hyperglycaemia being definitely associated with the appearance of convulsions. As to the mechanism which produces the rise in blood-sugar, there is no satisfactory evidence. The most probable supposition is that a nervous stimulation occurs, producing adrenaline secretion and consequent mobilisation of glycogen. The absence of a detectable increase in blood-glucose in the rat during convulsions is probably to be explained by depletion of its carbohydrate stores. Kinnersley and Peters [1930] have recently reported finding a local production of lactic acid in the brain of vitamin B-deficient pigeons. If that lactic acid is a cause and not an effect of local stimulation, it may conceivably also be the cause of a *piqûre* type of effect giving rise to adrenaline secretion.

Finally, the author is not of the opinion that the finding of the hyperglycaemia throws any light on the essential nature of vitamin B₁-deficiency. It appears to be a subsidiary effect; the birds are definitely not well before they show the hyperglycaemia; the cause of failure to grow and to maintain health is not explained by it.

SUMMARY.

1. A comparison between the Hagedorn-Jensen and the Somogyi methods of zinc hydroxide precipitation in human blood gave an average value of 8 mg. higher reduction with the former than with the latter.

2. A similar comparison on pigeons' blood gave values 16-32 mg. higher with the former than with the latter.

3. Using Folin-Wu, Somogyi and Herbert and Bourne filtrates, "total sugar," "true sugar," and non-sugar reducing values for fasting, adult pigeons' blood have averaged respectively 243, 182, 62 mg.

4. The isotonic filtrate of Herbert and Bourne gives practically the same values as the Somogyi filtrate for birds' blood.

5. There is a marked rise in "true sugar" during the convulsive stage in vitamin B₁-deficient pigeons.

6. No increase was found in the non-sugar reducing substances or cell volume of the blood of vitamin B₁-deficient pigeons or rats.

7. No increase was found in the "true sugar" of the blood of vitamin B₁-deficient rats.

8. There is usually a fall in blood-sugar in the final stages of vitamin B₁ deficiency in rats, but pigeons dying from convulsions or from hydro-pericardium are often hyperglycaemic.

9. When blood is obtained from the hearts of rats which have been rapidly killed by dislocation of the neck, it shows an increased sugar content in most

cases compared with circulating blood obtained immediately beforehand from the tail.

10. The hyperglycaemia in pigeons is thought to be the result of nervous stimulation—something of the nature of the hyperglycaemia of asphyxia, anaesthesia or diabetic *piqûre*. Its failure to occur in convulsive rats is ascribed to exhaustion of glycogen stores.

I am indebted to the Royal Society of Medicine for the scholarship which has enabled me to carry out this research, and to the Medical Research Council for a grant to defray the cost of materials.

I wish to thank Prof. Drummond for helpful advice and criticism.

REFERENCES.

- Cori (1931). *Physiol. Reviews*, **11**, 147.
Dale and Evans (1920). *J. Physiol.* **54**, 167.
Drummond and Marrian (1926). *Biochem. J.* **20**, 1245.
Duggan and Scott (1926). *J. Biol. Chem.* **20**, 1229.
Ege and Roche (1930). *Scand. Arch. Physiol.* **59**, 75.
Folin (1928). *J. Biol. Chem.* **77**, 421.
—— (1930). *J. Biol. Chem.* **86**, 173.
—— and Denis (1913). *J. Biol. Chem.* **14**, 29.
—— and Wu (1919). *J. Biol. Chem.* **38**, 106.
Funk and Schönborn (1914). *Z. physiol. Chem.* **89**, 373.
Gulland and Peters (1930). *Biochem. J.* **24**, 91.
Hagedorn and Jensen (1923, 1). *Biochem. Z.* **135**, 46.
—— (1923, 2). *Biochem. Z.* **137**, 92.
Herbert and Bourne (1930, 1). *Biochem. J.* **24**, 299.
—— (1930, 2). *Biochem. J.* **24**, 1787.
—— and Groen (1930). *Biochem. J.* **24**, 291.
Holden (1926). *Biochem. J.* **20**, 263.
Kinnersley and Peters (1930). *Biochem. J.* **24**, 711.
Kramer and Steiner (1931). *Biochem. J.* **25**, 161.
Kon and Drummond (1927). *Biochem. J.* **21**, 633.
Macleod (1926). Carbohydrate metabolism and insulin, p. 234.
(Longmans, London.)
Somogyi, M. (1928). *Proc. Soc. Exp. Biol. Med.* **26**, 353.
—— (1930). *J. Biol. Chem.* **86**, 655.
Scheunert and Pelchrzim (1923). *Biochem. Z.* **139**, 17.
Sure and Smith (1929). *J. Biol. Chem.* **84**, 727.