CCC. A NOTE ON THE LEVEL OF CARBONYL COMPOUNDS IN HUMAN BLOOD.

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IN view of the results obtained by Thompson and Johnson [1935] for the level of pyruvic acid in the blood of normal and vitamin B₁-deficient pigeons and rats it was of interest to investigate the level of this compound in human blood. Thompson and Johnson showed that the concentration of pyruvic acid in the blood of avitaminous pigeons was very substantially higher than the normal level; the figures obtained were, for the normal, 0.84 mg. of pyruvic acid per 100 g. of blood, and for the avitaminous 5.65 mg. per 100 g. of blood, as estimated by the extraction of the 2:4-dinitrophenylhydrazones [see Case, 1932; Peters and Thompson, 1934].

The following communication is a note on the level of the total bisulphite-binding substances present in human blood. As in the previous work with animals, bisulphite-iodine titrations were first carried out on the blood filtrates before embarking on the more elaborate method of extraction of the hydrazones. In this work on human blood however bisulphite-iodine titration gave no indication of raised blood-pyruvate levels in any of the diseased conditions examined, so that hydrazone extractions have not been carried out. Several attempts were made to apply the full method of Clift and Cook [1932] for determining pyruvic acid, but in every case it was found that the titre was increased on heating, owing presumably to the formation of bisulphite-binding compounds from the sugars and amino-acids present, since Engfeldt [1920] observed that the amino-acids in blood filtrates break down to form acetone when heated in alkaline solution. It was also found that trichloroacetic acid (used as the protein precipitant) produced bisulphite-binding substances on heating: Merck's, Kahlbaum's and B.D.H. samples all gave this effect. For these reasons the titrations were limited to estimations of the total bisulphite-binding capacity of the blood filtrates.

Method. The method employed was as follows. Venepuncture was made with a medium-sized needle into one of the arm veins. About 3 ml. of blood were allowed to drip into a weighed centrifuge-tube containing 3 ml. of 25 % trichloroacetic acid and 4 ml. of water. (In some experiments a syringe was used, and the blood was squirted into the tube.) The tube was then re-weighed and the precipitate re-extracted in the same way as described for pigeon's blood. The combined centrifugates were then brought to p_H 2.0 with 20 % sodium hydroxide, made up to 25 ml. and titrated for bisulphite-binding capacity.

Results. The results obtained for normal blood are given in Table I.

From these figures it may be seen that the results obtained for normal blood fall within strikingly narrow limits. The centrifugates were also tested for the presence of acetone bodies by means of the nitroprusside reaction, but in none

Table I. *Bisulphite-binding capacity of normal blood.*

(Expressed as mg. pyruvic acid per 100 g. blood.)

Exp.	Sex	B.B.S.	Exp.	Sex	B.B.S.
105	F	2.19	140	M	2.71
126	M	2.35	141	M	2.91
131	F	3.13	144	M	2.71
146	M	2.90	158	F	2.79
133	M	3.30	161	M	2.45
134	M	1.96	162	M	2.48
135	M	4.00	163	M	2.52
136	M	2.34	164	M	3.17
137	M	2.86	165	M	3.12
138	M	2.10	166	M	2.56
139	M	2.48	167	M	3.69

Average 2.81

of these cases was a positive result obtained, so that no very high concentration of these substances can have been present. Furthermore, the careful work of Engfeldt [1920] provides an upper limit for the level of acetone bodies in the blood of normal human subjects in terms of $N/100$ iodine, which is of particular interest when reviewed in connection with our figures for the iodine titration of the total carbonyl compounds present. Engfeldt found, from his titrations of acetone bodies, that 2 ml. of normal human blood never have a titration of more than 0.05 ml. $N/100$ iodine, *i.e.* 100 ml. of normal blood have a maximum content of acetone bodies equivalent only to 2.5 ml. $N/100$ iodine.

If we now make use of the figures obtained by Arndt [1921] for the specific gravity of whole blood (1.02), we find that the upper limit of the level of acetone bodies in normal blood, according to Engfeldt's figures, is equivalent to 2.45 ml. $N/100$ iodine per 100 g. of blood. The average level presented above for the total bisulphite-binding capacity of human blood when reconverted into iodine values is equivalent to 6.39 ml. $N/100$ iodine per 100 g. of blood. We find therefore a concentration of carbonyl compounds in normal human blood, over and above that of the acetone bodies, equivalent to 3.94 ml. $N/100$ iodine per 100 g. of blood, or expressed in terms of pyruvic acid, 1.73 mg. per 100 g. blood. Moreover, it must be remembered that Engfeldt's figures include β -hydroxybutyric acid, which is not estimated by our method, so that the level of carbonyl compounds over and above that of acetone and acetoacetic acid is, in reality, slightly higher than the figure given above.

Although hydrazone extractions have not been carried out, the nitroprusside reaction of Simon and Piaux [1924] has been watched in every experiment, but in none of the experiments on normal blood did a positive reaction develop. On working out the lower limit of sensitivity of this reaction, however, we have found that this agreed with our level of "non-acetone body" carbonyl compounds calculated from Engfeldt's results. The lower limit of the reaction was found by us to be given by a concentration of pyruvic acid of about 2γ per ml., and a concentration of this order in the final centrifugates in which the reaction was carried out would correspond to a level of 1.64 mg. per 100 g. of blood, whilst our level of "non-acetone body" carbonyl compounds was found to be only 1.73 mg. per 100 g. of blood. On the other hand it must be remembered that this level found by us is, owing to the inclusion of β -hydroxybutyric acid in Engfeldt's figures, in all probability too low, so that it seems probable that there may exist in normal human blood detectable amounts of some carbonyl compound other than pyruvic acid, acetone, or acetoacetic acid.

In view of the known relationship between pyruvic acid and brain metabolism [Peters and Thompson, 1934] estimations were also done on the blood of patients suffering from various forms of insanity (see Appendix), but although in several instances high values were obtained it was impossible to conclude that in any of the conditions examined was there any statistical difference from the normal level.

The blood was also examined from a number of hospital patients suffering from physical disease (see Appendix), including some whose conditions were suggestive from the point of view of their symptomatology of a vitamin B₁ deficiency, but here also no true evidence of a raised level was obtained. We have been unable to procure any cases of beri-beri for inclusion in our results, but we are of the opinion that it is of value in view of future work on this condition to publish these results obtained with normal blood.

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APPENDIX.

The following table shows the results obtained on the blood of patients suffering from mental and physical disease. As already mentioned the figures show no significantly raised level of bisulphite-binding substances, and are merely included in order to show the variations of the values obtained.

Bisulphite-binding substances in the blood in disease.

(Expressed as mg. pyruvic acid per 100 g. blood.)

1. *Mental disease:*

Exp.	Sex	Age	Diagnosis	B.B.S.
78	M	25	Epilepsy	2.02
79	M	24	Hysteria (cured?)	3.58
80	M	—	Schizophrenia	2.52
81	M	75	Manic depressive psychosis	4.20
82	M	50	General paralysis	2.06
83	M	30	Chronic encephalitis	2.57
84	M	30	Schizophrenia	2.78
85	M	30	Schizophrenia	2.74
86	M	16	Hebephrenia	5.90
115	M	16	Same patient, 2 weeks later	4.28
87	M	50	Dementia, pernicious anaemia	2.74
88	M	—	Schizophrenia	3.77
90	F	10	Cretinism	2.38
91	M	6	Cretinism	3.11
92	F	13	Cretinism	4.35
98	M	—	Senile dementia	1.73
99	M	—	Senile dementia	3.90
100	M	—	General paralysis	3.66
111	M	15	Mental deficiency	3.34
102	M	15	Mental deficiency	2.48
103	F	—	Senile dementia	2.00
104	F	—	Acute melancholia	2.90
114	M	72	Senile dementia	3.68
117	F	60	Acute melancholia	3.35
118	F	40	Manic depressive psychosis	4.16
119	F	50	Manic depressive psychosis	3.14
122	F	—	Acute melancholia	3.89
124	F	43	Schizophrenia, Cushing's syndrome	1.60
125	F	38	General paralysis	1.81

2. *Physical disease:*

Exp.	Sex	Age	Diagnosis	B.B.S.
61	M	50	Osteitis deformans	1.78
62	M	60	Osteitis deformans	1.66
106	M	—	Sciatica	2.09
107	M	—	Post-diphtheritic polyneuritis	2.44
108	M	70	Asthma	5.31
109	M	65	Carcinoma of oesophagus	3.90
142	M	—	Polycythaemia rubra vera	3.98
143	F	—	Hyperthyroidism	4.80
147	F	70	Undiagnosed	2.85
148	M	—	Cerebral tumour	2.46
156	F	48	Hemiplegia	3.02
158	F	33	Sciatica and jaundice	4.44
159	F	46	Undiagnosed	2.79
160	F	33	Hyperthyroidism	3.14
168	F	—	Hyperthyroidism	2.30
169	M	—	Hirschsprung's disease	2.58
170	M	—	Hyperthyroidism	2.15
171	M	64	Sarcoma of femur	6.65
172	M	59	Carcinoma of rectum	2.75
173	F	—	Pregnancy toxæmia	4.12
174	F	—	Pregnancy toxæmia	4.40
175	M	—	Carcinoma of bladder	3.03
176	M	—	Multiple fibrosarcomata	2.39

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