THE EFFECT OF COBALT ON THE OXYGEN CAPACITY AND THE METHEMOGLOBIN CONTENT OF THE BLOOD

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ONE THEORY as to the mechanism of the production of polycythemia in the rat, and several other species, by cobalt, is that this substance interferes with cellular oxidative processes.[1-4] One way in which such an effect might be produced would be by an interference with the transport of oxygen in the blood to the cells, either by a decrease in the oxygen capacity of hemoglobin itself, or by the formation of "methemoglobin" possibly containing cobalt in place of iron. In the present investigation, a study was made of the oxygen capacity and the methemoglobin and cobalt content of the blood of rats which had been maintained in a state of polycythemia by cobalt administration for a period of at least six weeks.

Experimental

Male, weanling, albino rats, Connecticut Agricultural Experimental Station strain, were used. They were fed an adequate synthetic basal diet described in a previous publication.[2] Various supplements were added to the basal diet in amounts also described in detail in the above paper. The groups studied included a control group and groups given cobalt alone (477 mg. recrystallized $CoSO_4 \cdot 7H_2O$ per kilo of diet), or cobalt supplemented with either choline (2.0 Gm. choline chloride per kilo of diet), or cysteine (1.56 Gm. L cysteine hydrochloride per kilo of diet). The latter two groups of animals were part of a different study to be reported later. The amount of cobalt sulfate added to the diet supplies each rat with approximately 1.0 mg. cobalt per day, an amount found in previous studies to produce a definite polycythemia in the rat. After the animals had been on experiment for a period of twenty weeks and the cobalt-treated rats had developed the characteristic polycythemia, with the exception of those given cysteine, as will be described in a subsequent publication, they were sacrificed and samples of blood were taken for analysis in the following manner. The animals were anesthetized with ether and five to eight ml. of blood was drawn from the heart into tubes containing heparin. Of this amount, a very small portion was used for the "total" hemoglobin determination by the acid hematin method using the Coleman spectrophotometer. One ml. was used for the determination of oxygen capacity, 1 ml. for the estimation of methemoglobin, and the remainder was reserved for a spectrographic analysis* for cobalt. The method used for the determination of the oxygen capacity of the blood was Sendroy's modification using the Van Slyke-Neill manometric appa-

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ratus.[5] The methemoglobin content of the samples was determined by a colorimetric procedure outlined in Kolmer and Boerner.[3] The remainder of the blood was carefully ashed and a spectrographic analysis for cobalt was made.

Results and Discussion

In Tables 1 and 2 are recorded the average terminal hemoglobin values, as determined by the acid-hematin and oxygen-capacity methods, and the average methemoglobin content of the blood of the various groups of animals. It is evident from the data in the two tables that there is a close correlation between the hemoglobin values by the two methods and that there is no significant amount of methemoglobin present in the blood of the cobalt-treated rats. The greater variations from

Table 1.—*Terminal Hemoglobin Values of Control and of Cobalt-treated Rats*

Group	Number of rats	Hemoglobin—Gm. %			
		Acid Hematin Method		O_2 Capacity Method	
		Average	Standard Deviation	Average	Standard Deviation
Control	8	15.4	±0.4	15.5	±0.5
Cobalt	8	19.7	±1.9	19.7	±2.1
Cobalt + Choline	7	19.2	±1.4	19.6	±1.3
Cobalt + Cysteine	8	17.4	±1.7	17.1	±1.5

Table 2.—*Methemoglobin Content of Blood of Control and Cobalt-treated Rats*

Group	Number of Rats	Total Hemoglobin Average Gm. %	"Active" Hemoglobin (by O_2 Capacity) Gm. %	Methemoglobin	
				Gm. %	Range
Control	8	15.3	15.6	−0.3	0.0 to −1.3
Cobalt	8	20.8	19.6	+1.2	+2.8 to −1.9
Cobalt + Choline	7	19.2	19.6	−0.4	+1.4 to −2.6
Cobalt + Cysteine	7	18.7	17.1	+1.6	+2.2 to 0.0

the average in the terminal hemoglobin and methemoglobin values observed in the cobalt-treated rats, as compared with the controls, appears to be a result of greater difficulties in obtaining and measuring blood samples in the former groups. The blood of the rats given cobalt was extremely viscous. The spectrographic analyses of the ashed blood samples showed no more than trace amounts of cobalt in any specimen.

The foregoing observations together thus constitute evidence that the mechanism of the production of polycythemia by cobalt is not one of the formation of an altered type of hemoglobin having a decreased oxygen-carrying capacity, nor can it be attributed to the formation of methemoglobin. Further substantiation of this view is afforded by the results obtained in the spectrographic analysis for cobalt which demonstrated the absence of more than a trace of that element in the blood. This latter observation is in agreement with that of Stare and Elvehjem[6] who found

only traces of cobalt in the blood of cobalt-treated polycythemic rats by a colorimetric method using nitroso-R-salt.

Conclusions

A study has been made of the oxygen capacity and the methemoglobin and cobalt content of the blood of rats administered approximately 1 mg. cobalt daily for twenty weeks, in order to produce a sustained polycythemia.

No evidence of a decrease in the oxygen capacity of the blood of the cobalt-treated polycythemic rats was found, nor did the methemoglobin content differ significantly from the small amount found in the blood of control rats. No more than traces of cobalt were found in the blood of either group by spectrographic analysis.

These observations are interpreted as evidence that the mechanism of the production of polycythemia by cobalt is not one of lowering the oxygen capacity of hemoglobin nor of producing a methemoglobin, possibly containing cobalt rather than iron in the hemoglobin molecule.

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