Physiology. — "*Concerning the Influence of the Administration of Iron on the Respiration of the Red Bloodcorpuscles.*" By Prof. A. A. HIJMANS VAN DEN BERGH and M. J. ROESSINGH."

(Communicated at the meeting of December 27, 1923).

It is well-known that the haemoglobin-containing red bloodcorpuscles are the carriers of the oxygen of the atmosphere, required for the oxidative processes of the tissue cells. MORAWITZ was the first to ascertain whether the red bloodcorpuscles have a respiration of their own. The author demonstrated that in man the oxygen-consumption of the red bloodcorpuscles is, under normal conditions, very trifling, amounting to some 3 or 4 %. This lack of respiration is in keeping with other properties of these cells: absence of a nucleus, absence of the function of reproduction, which allot to them a place different from that of all living cells and induce us to consider them as an intermediate form between dead and living matter.

Contrary to what we observe in man and in mammals the oxygen-consumption of the red bloodcorpuscles in birds is of considerable importance. The difference is apparently due to the human erythrocytes being non-nucleated, whereas the red cells in birds, as is well-known, contain a nucleus. That, however, this view is not quite correct, is borne out by further experiments made by MORAWITZ in collaboration with MASING. The oxygen-consumption of the red bloodcorpuscles of mammalia is indeed largely increased through withdrawal of blood (bloodletting), and in general through all sorts of operations (administration of blooddestroying poisons, such as phenylhydrazin) which make the animal anaemic and bring about a strong regeneration of young bloodcorpuscles. In these cases, however, no nucleated red cells will enter the peripheral blood, or, if they do, their number is so small, that they cannot be responsible for the increase of the oxygen consumption. It is generally received that the increased oxygen consumption in these circumstances is owing to the youthful state of the red cells. Probably the oxidative processes are related to the presence of nuclear fragments in these cells, although they are not demonstrable under the microscope, or to their chemical composition, which is supposed to be different in the young and the old corpuscles. Just as in the animal experiment the human blood

also appears to have an increased oxygen consumption, whenever the bloodbuilding organs are in a state of active bloodformation. Thus we find an increased O consumption in pernicious anaemia, and in the blood of individuals regenerating actively, as in all sorts of intoxications and after hemorrhage, and in general with excessive activity of the bone marrow.

On a previous occasion Dr. ROESSINGH has in my laboratory tried to ascertain by this method whether the anaemia that is often met with in carcinoma, occurs with an active regeneration of red blood-corpuscles.

The result was negative: The O consumption of the blood of individuals with carcinoma was not greater than in normal men. On the other hand ROESSINGH found in pernicious anaemia, and in the blood of individuals suffering from other forms of anaemia, and also shortly after bloodtransfusion, considerable increase (instead of the normal value 3—4 %: some 10 to 60 %).

By means of the determination of the O consumption of the red blood cells ROESSINGH has recently studied the effect of iron on bloodformation.

Anaemic patients were given large doses of iron during several weeks. Before and after treatment the oxygen consumption of the red blood-corpuscles was measured.

The defibrinated blood is centrifugalized; then the red blood-corpuscles are washed out with a physiological NaCl-solution in order to remove the serum. Now the emulsion of blood-corpuscles is shaken up with air for saturation of the haemoglobin with oxygen. Particular attention should be given to absolute sterility, because many bacteria have a considerable consumption of oxygen, which, if disregarded, must lead to grave errors. After the haemoglobin has been saturated with O, the quantity of loosely combined oxygen in a portion of the blood is determined after the method of HALDANE in a BARCROFT apparatus. Another portion of the blood is placed for four hours in the incubator at 37°, being well shut off from the air; after this a determination of the loosely bound oxygen in the second blood-portion is made. The difference in the amount of the loosely bound oxygen before and after the incubation represents the oxygen withdrawn by the corpuscles from their own haemoglobin for their own respiratory function.

The results of this inquiry have been tabulated below. The different blood samples apparently behave in a different way, which we have not been able to account for. In one case the O.-consumption after Fe-administration appeared to be diminished, which is most likely

due to an experimental error. In another experiment it remained constant, but in the majority of cases a considerable augmentation was noted, in consequence of, or anyhow subsequent to the Fe-administration. In these cases we are inclined to conclude that the iron has stimulated the bonemarrow to an active formation of young cells.

However, we have to discuss an objection. It is well-known that in many biochemical processes iron is believed to play the part of a catalyst. It might be asked whether the iron may perhaps be combined in the molecule of the corpuscles, thus inciting respiration. This hypothesis deserves the more consideration in view of WARBURG's observation that the sea-urchin eggs consume more oxygen and give off more CO_2 after the addition of highly diluted ferro-, or ferri-salts (60—100 %) than without this addition. In order to test this objection Dr. ROESSINGH has added dilute iron-salts to emulsions of red blood-corpuscles and examined the O-consumption before and after the addition. The Fe-salts proved to exert no influence.

For the present we may therefore conclude that in all probability large doses of iron administered to anaemic patients stimulate the bloodbuilding organs, so as to enable them to supply the peripheral blood with young red bloodcells.

We are tempted to compare the oxygen consumption of the red bloodcorpuscles with glycolysis. This process, thoroughly studied by LÉPINE, has occupied the attention of a great number of researchers. Many have called in question LÉPINE's conclusions or have denied their great importance, so that the subject was dropped. Not until quite recently, it has again been studied with much interest.

We wanted to know whether glycolysis, by which is meant the spontaneous disappearance of glucose from the blood, has anything to do with the internal respiration of the red bloodcorpuscles. When studying this problem in my laboratory, Dr. VAN STEENIS found, contrary to the current opinion, that the red bloodcorpuscles in man possess a glycolytic function. We then ascertained whether the presence of oxygen has a considerable influence upon this process. To this end two series of experiments were compared. In one series the fluid was shut off from the air. In the other we allowed oxygen to pass through the fluid. We found no differences. Ultimately we compared glycolysis of bird's blood and human blood. If there were any relation between glycolysis and the oxygen-consumption of the red bloodcorpuscles a higher degree of glycolysis might be expected in bird's blood than in human blood, since indeed the internal respiration in birds is much more considerable than in man. Such a

difference we failed to detect. We, therefore, feel justified in concluding that the two processes are not related.

	Before the admin. of iron			*After* the admin. of iron		
	Haemogl. content	Red blood-corpuscl.	Oxygen consump.	Haemogl. content.	Red blood-corpuscl.	Oxygen consump
1. *R.* Chron. nephritis. 30 days; 2.25 gr. Fe daily			14.66 %			72.04 %
2. *Miss V.* Anaemia after hemorrhage. 20 days; 2.25 gr. Fe daily	70 %	4.400000	none			13.10 %
3. *v. d. H.* Tuberc. pulmonum. 30 days; 2.25 gr. Fe daily			45.66 %			41 %
4. *Miss D.* Chron.nephritis. 14 days; 2.25 gr. Fe daily	42 %	1.860000	none	52 %	2.200000	46.33 %
5. *Miss deL.* Splenomegaly. 30 days; 2.25 gr. Fe daily	105 %	4.000000	2.44 %	100 %	4.400000	51.28 %
6. *Miss S.* Tumor mediastini. 18 days; 2.25 gr. Fe daily	90 %	4.200000	11.30 %	85 %	3.800000	28.00 %
7. *Miss Z.* Purpura hemorrhage. 18 days; 2.25 gr. Fe daily	70 %	3.600000	none	85 %	3.850000	57.72 %
8. *v. E.* Malignant abdominal tumor. 18 days; 2.25 gr. Fe daily	55 %	3.800000	12.67 %	50 %	4.440000	22.40 %
9. *Miss v. d. H.* Adipositas. 25 days; 2.25 gr. Fe daily	48 %	3.500000	none	64 %	3.800000	16.27 %
10. *Miss v. D.* Tuberc. peritonei. 46 days; 2.25 gr. Fe daily	64 %	4.200000	none	82 %	4.510000	3.73 %
11. *Miss D.* Colitis. 22 days; 200 mgr. Fe daily	48 %	3.180000	5.54 %	55 %	4.200000	38.97 %
12. *H.* Sacroma lymphatic glands 21 days; 2.25 gr. Fe daily	100 %	5.120000	4.27 %	98 %		2.12 %

Continued.

	Before the admin. of iron.			*After* the admin. of iron.		
	Haemogl. content.	Red blood-corp.	Oxygen consump.	Haemogl. content.	Red blood corp.	Oxygen consump.
13. *de B.* Tuberc. abscess. 21 days; 2.25 gr. Fe daily	85 %	4.900000	none	85 %	4.780000	7.63 %
14. *B.* Otitis chronica. 21 days; 2.25 gr. Fe daily	100 %	5.170000	none	95 %	5.000000	none
15. *v. Z.* Pernic. anaemia. 21 days; 2.25 gr. Fe daily	48 %	1.370000	none	45 %	1.530000	10.09 %
16. *L.* Carcin. ventriculi. 14 days; 2.25 gr. Fe daily	31 %	3.170000	none	32 %	2.360000	none
17. *Miss B.* Anaemia. 19 days; 2.25 gr. Fe daily	43 %	3.120000	44 %	60 %	4.400000	none
18. *Miss C.* Spondylitis. 21 days; 2.25 gr. Fe daily	100 %		none	100 %		none
19. *W.* Anaemia. 25 days; 2.25 gr. Fe daily	50 %	4.400000	none	62 %	5.040000	52.13 %
20. *Miss V.* Arteriosclerosis. 21 days; 300 mgr. Fe daily	95 %		4.21 %	95 %		none
21. *Miss G.* Apoplexy. 21 days; 2.25 gr. Fe daily	95 %		none	100 %		22.25 %
22. *Miss V.* Diabetes. 21 days; 1.5 gr. Fe daily	88 %	4.530000	21.31 %			23.82 %
23. *Miss E.* Anaemia. 23 days; 2.25 gr. Fe daily	54 %	4.400000	11.7 %	88 %	4.250000	27.25 %