

Medicine. — *Hypophysis and bloodpicture VI. Direct arguments for the blooddemolition-theory.* By G. A. OVERBEEK and P. RUITINGA Jr. (From the department of Pharmacology of the University of Leiden (Holland). Director: Prof. Dr. S. E. DE JONGH). (Communicated by Prof. J. VAN DER HOEVE).

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It has become clear from earlier investigations, described in this series of papers, (1. 2. 3. 4. 5.,) that the influence of the hypophysis on the red blood picture is not caused by a direct stimulation of the bone marrow but rather by an augmentation of the processes of erythrocyt-destruction. The arguments given in favour of this theory, though strong enough, always bore an indirect character except the one about the influence on the urobilin excretion. The latter investigation, however, was seriously hindered by technical difficulties, so that the results were less impressive than we hoped them to be. Therefore, we sought and found further direct arguments for our blooddemolition-theory, which will be described in this paper. They are: I. the production of anemia and II. an influence on the bilirubin excretion with the bile after the injection of pituitary extract.

I. If pituitary extracts should contain a substance, promoting blood destruction, one might expect an anemia after treatment with high dosages. In our earlier work we found indications that our injected animals had less erythrocytes than the controls, especially if these controls were injected with boiled pituitary extract, which tends to augment the number of erythrocytes.

After isoelectrical purification of crude alkaline anterior lobe extract (for technical details see the preparation of Y extract, RUITINGA (5)) normal rabbits as well as hypophysectomized rats could be rendered anemic by injection of the new product (table I). This anemia was always accompanied by a strong reticulocytosis. As the number of reticulocytes was much higher than obtained by the injection of a crude extract and often correlated with the seriousness of the anemia (in many cases the anemia became manifest, when the reticulocyte level surmounted 10 %), we think it quite clear that the influence on the reticulocytes must be seen as the consequence of a diminution of the erythrocytes.

One might ask, what could be the cause of the augmented activity of the purified extract. We supposed that the effect was caused by the elimination

TABLE I.
Anemia in rabbits and rats after the injection of pituitary extract.

Number of animal	Erythrocytes mill./mm ³		Reticulocytes ‰		Remarks
	Initial value	Lowest value	Initial value	Highest value	
					Rabbit
D 632	—	1.6	—	320	1 × 2.5 cc Y extr. for 5 d.
D 633	5.1	3.7	2	60	ibid. 8
—	5.0	4.1	6	6	ibid. 10
—	5.5	4.0	4	40	ibid. 13
					Hyp. ect. rat
B 5484	6.9	4.1	2	220	2 × 0.5 cc Y-extr. for 14 d.
B 5485	6.9	4.0	8	100	ibid.
B 5487	6.6	5.2	2	120	ibid.
B 6049	6.5	6.2	72	120	ibid.
B 6050	6.6	3.9	48	400	ibid.
B 6051	5.9	5.9	28	72	ibid.
B 6052	7.3	5.7	24	104	ibid.

Note: In some other hypophysectomized rats erythrocyte values were observed of 3.0; 3.7; 4.3 and 3.8 mill./mm³. As we have no initial values of these animals at our disposition, they are not included in the table, though they may be considered as definitely anemic.

of a factor influencing blood *formation*. Such a factor was described by FLAKS et al. (6). These investigators succeeded in producing a polycythemia in normal rats by the *oral* administration of anterior lobe extract. They never observed any effect after the *injection* of their extracts. As it is not very likely that this substance would show its activity after *oral* administration only, and knowing that crude extracts stimulate blood destruction when *injected*, it becomes probable that the substances described by FLAKS and by us are both present in crude extract. The activity of these crude extracts cannot be very great in either respect, the substances counteracting each others activity.

If this were true, it should be possible to produce a polycythemia by *injecting* the fraction remaining after the preparation of the Y extract.

Although the results were not overwhelming, sometimes a slight polycythemia could be shown after the administration of this extract. In two out of nine rats the erythrocytes increased from 8 million/mm³ to more than

10 millions/mm³, this being the level above which FLAKS et al. consider a polycythemia to be present.

In other animals increases to more than nine millions/mm³ were observed. This result therefore indicates the existence of two substances in the anterior lobe of the hypophysis both stimulating the bone marrow, but acting in a different way. One of them acts in a direct way (FLAKS) the other indirectly by promoting blood destruction.

II. The excretion of bilirubin with the bile was studied in the following way:

A rat was anaesthetized with urethane, and the duodenum was ligated by the pylorus after washing with about 5 cc. of saline. The duodenum was emptied by gentle digital pressure and a second ligature was made about 7 cm more distally. In this way the bile-duct opens into the ligated intestinal loop. The peritoneum and the skin were closed, and the animal was put away for one hour. Consecutively the ligated loop was taken from the animal and the contents brought into a small centrifuging glass. After centrifuging, the clear yellow liquid was sucked up in a syringe, the amount was measured and the whole diluted with water to 10 cc.

The yellow colour was compared with the colour of diluted bile of a normal rat arbitrarily chosen as a standard (100). The yellow colour must originate from bilirubin only, as it was impossible to bring the yellow substance in chloroform, unless the mixture was acidified with strong hydrochloric acid, after which the yellow substance passed *quantitatively* into the chloroform. We used this rather primitive test method for the estimation of bilirubin because the usual methods failed with rats' bile (HYMANS VAN DEN BERGH test, method of SABATINI). The latter method which was designed for the estimation of bilirubin in urine was also tried after addition of diluted urine to the bile. Some colour was obtained, but for our purpose the method was not satisfactory in a quantitative sense, so that we stucked to the primitive method described above.

The use of a rat as a "standard rat" made it impossible to compare the results directly. Therefore we calculated the percentage of difference between each hypophysectomized rat and each normal rat in every group, in this way eliminating the influence of the accidental choice of the "standard rat". These percentages of difference could be averaged for the different groups.

In a first experimental series, hypophysectomized rats (about 10 days after the operation) were compared with normal rats. Table II shows that in all operated animals the reticulocyte level was low. In almost all instances the bilirubin excretion was diminished, although the amount of bile was not significantly different. The average percentage of decrease, calculated as mentioned before, amounted to 31.7 %.

In a second series hypophysectomized rats treated with crude pituitary extract were compared with normal rats. Table III proves that the number of reticulocytes was normal or above normal and that the excretion of

bilirubin was certainly not less than in the normal rats. There even was an average increase of 18.5 % compared with normal.

Thus it could be shown that hypophysectomized rats excrete less bilirubin than normal rats, and that this failure can be corrected by the injection of crude pituitary extract.

These results are in complete accordance with the theory that blood-demolition is diminished in the absence of the hypophysis.

TABLE II.
Bilirubin excretion in normal and hypophysectomized rats.

Number of rat	Body weight on day of experiment	Retic. ‰ on day of experiment	cc of bile	Total bilirub.	Days after operation	Remarks
B 7153	95	0	0.35	60	9	Hyp. ect.
B 7154	126	4	0.60	73	9	" "
B 7155	111	36	0.40	105	—	Normal
B 7156	93	68	0.45	100	—	"
B 7157	120	2	0.40	35	10	Hyp. ect.
B 7158	116	4	0.60	64	10	" "
B 7159	142	36	0.36	94	—	Normal
B 7160	110	20	0.40	100	—	"
B 7161	123	0	0.25	33.5	10	Hyp. ect.
B 7162	99	2	0.22	36.5	10	" "
B 7163	126	32	0.25	100	—	Normal
B 7164	95	72	0.22	46	—	"
B 7165	112	0	0.25	70	13	Hyp. ect.
B 7166	132	0	0.22	65	13	" "
B 7242	112	2	0.25	66	13	" "
B 7243	115	44	0.33	61	—	Normal
B 7244	133	60	0.52	100	—	"
B 7245	134	0	0.56	86	13	Hyp. ect.
B 7246	100	0	0.36	61	13	" "
B 7247	94	48	0.35	104	—	Normal
B 7248	96	72	0.28	100	—	"

TABLE III.

Bilirubin excretion in normal and hypophysectomized rats treated with pituitary extract.

Number of rat	Body weight on day of experiment	Retic. $\frac{0}{100}$ on day of experiment	cc of bile	Total bilirub.	Days after operation	Remarks
B 7102	186	108	0.60	185	15	Hyp. ect.
B 7103	176	100	0.35	115	15	" "
—	140	44	0.50	100	—	Normal
—	180	100	0.40	115	—	"
B 7223	131	44	0.33	108	9	Hyp. ect.
B 7224	114	56	0.39	119	9	" "
B 7225	135	64	0.42	117	9	" "
B 7261	146	76	0.30	100	—	Normal
B 7262	114	52	0.16	86	—	"
B 7263	106	120	0.20	79	—	"
B 7226	132	72	0.60	64	11	Hyp. ect.
B 7227	131	136	0.40	66	11	" "
B 7228	142	92	0.58	76	11	" "
B 7264	154	60	0.31	100	—	Normal
B 7265	163	68	0.31	86	—	"
B 7266	150	32	0.35	49	—	"

The hypophysectomized rats were daily subcutaneously injected with 2×0.5 cc of crude alkaline anterior lobe extract.

Summary:

1. Crude alkaline pituitary extracts can be separated in two fractions, one promoting blood destruction and causing a hemolytic anemia, the other causing sometimes a slight polycythemia.

2. Hypophysectomized rats excrete less bilirubin with the bile than normal rats. After the injection of pituitary extract normal quantities are excreted.

These facts are *direct* arguments for the soundness of our theory that the hypophysis contains some factor promoting erythrocyte destruction.

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