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The Possible Relationship between the Pineal Gland and Red Cell Destruction by the Spleen *

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In a previous study (Pozzi and Barbolini) a possible mechanism, by which red cell destruction by the spleen was produced, in the rat was discussed.

The present study has given results which suggest that in this animal there may exist a relationship between the pineal gland and red cell destruction by the spleen.

Materials and Methods

The material consisted of 45 healthy, female, virgin Wistar rats, weighing between 140 and 160 g, which were divided into three groups.

Group I. 15 rats that were subjected to a control operation (opening of the cranial vault and section of the superior longitudinal sinus without removal of the pineal gland).

Group II. 15 rats that were subjected to resection of the pineal gland.

Group III. 15 rats that were subjected to resection of the pineal gland and were also given subcutaneous injections of a sterile solution of trypan blue (0,5%), beginning on the 61st day after the operation (5 injections of 2.5 ml every other day to a total of 62.5 mg).

The pineal glands were resected by the technique[1] of Thieblot and Le Bars. The animals were kept in separate cages, at room temperature (approx. 24° C), with normal daylight (approx. 12 hours per day), and fed a completely standardized diet with water *ad libitum*.

On the 70th day all the animals were killed by decapitation; serial sections of the epiphyseal region in Groups II and III confirmed that the resection of the pineal body was successful and that *no anatomical damage* had been caused to the related structures.

The spleens, which were removed immediately after death, were cut into two equal parts. One half was fixed in 10% calcium-formol and embedded in paraffin in the usual way. The other half was sectioned with a cryostat, and once the histo-enzymological tests had been completed, the sections were fixed prior to mounting with glycerin jelly.

The sections in paraffin were subjected to: silver impregnation by the method of Gomori, for the argentophil reticulum; the Prussian blue test for ferric iron; this was carried out both in the usual manner (Lison) and by the unpublished modified version of Mauri.

Mauri's *Modification.*

1. Bring paraffin sections to water.
2. Immerse in a satured solution of K ferrocyanide for 20 min.
3. Transfer to 1% solution of HCl in 70% alcohol.
4. Wash carefully in several baths of 70% alcohol.
5. Wash in distilled water.
6. If desired, counterstain briefly with haematoxylin-eosin.

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[1] The pinealectomy is carried out without introducing any surgical instrument into the brain. The pineal body is moved outside the third ventricle by means of traction applied to the point where the transverse sinus joins the longitudinal sinus (sectioned). The only damage caused is to the vascular tissue. The nature and extent of the damage was identical in all the rats (sham-operated and pinealectomized).

The cryostat sections were subjected to the following tests: Burstone's method (1) for alkaline phosphatase (30 min) with 0,2 M tris/HCl buffer at pH 8,3; Burstone's method (2) for acid phosphatase (30 min) with 0.2 M-acetate buffer at pH 5.4: the method of Balogh and Cohen for succinic dehydrogenase (30 min), TPN diaphorase (30 min), glucose-6-phosphate dehydrogenase (30 min), glutamate dehydrogenase (30 min) and DPN diaphorase (15 min) with 0.1 M-veronal buffer at pH 7.4.

For each enzymatic test, control preparations were made without the specific substrate. The reagents used were products of Sigma Chemical Co., Missouri, USA.

This investigation was carried out during the months of June to August.

Results

In the sham-operated rats the spleen showed a thin delicate argentophil reticulum. Both tests for ferric iron gave in all the animals a rather weak, granular

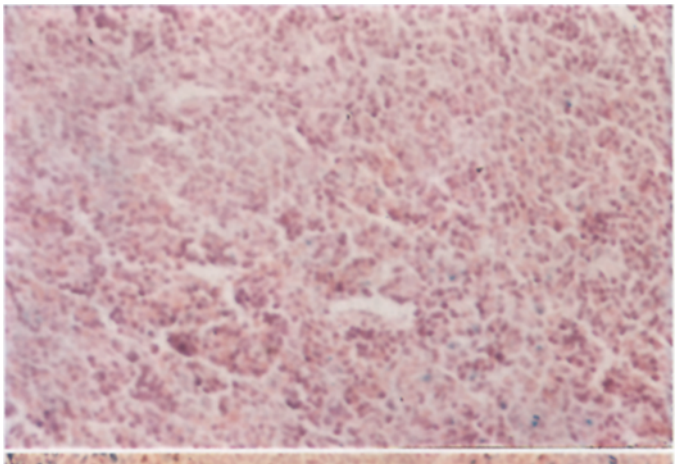

Fig. 1

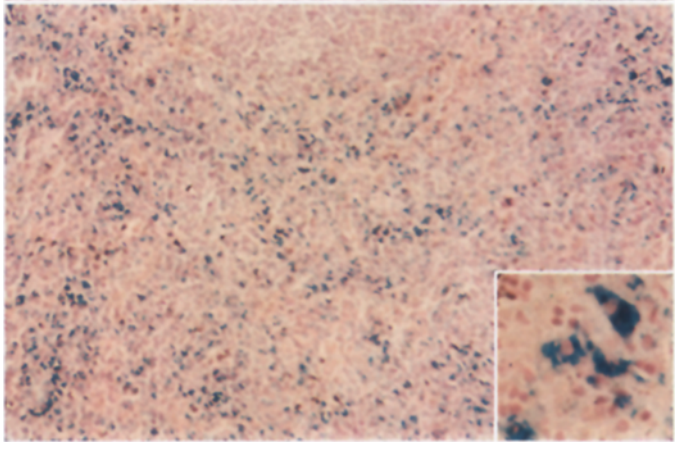

Fig. 2

Fig. 1. A weakly positive Prussian blue test in the cordonal histiocytes of rat subjected to sham operation.

Fig. 2. A strongly positive test in pinealectomized rat. Note the absence of the iron pigment in the histiocytes lining sinuses. × 125. In the detailed photograph the intracellular distribution can be seen.

reaction, localized in the histiocytes of the splenic cords (Fig. 1). The group of histiocytic enzymes observed was essentially the same as that in the normal rat

(POZZI and BARBOLINI). They were present in the histiocytes of the follicles and the cords of the spleen, but not in the histiocytes lining sinuses.

In the animals of Group II the argentophil network was coarser and more distinct.

With both techniques used, the reaction for ferric iron was very pronounced in all the rats, with minor variations from case to case; it was, however, definitely more marked than that observed in Group 1. The reaction appeared localized in the cytoplasm of histiocytes of Billroth cords, and occasionally in the histiocytes of spleen-follicles; it was never observed in the histiocytes lining sinuses.

The enzymatic activity of the histiocytes of the Malpighian follicles and of the trabeculae of the red pulp was the same or, more often, slightly more pronounced (acid phosphatase, TPN diaphorase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase) than in the controls of the first group.

The treatment with trypan blue brought about a slight but constant increase in the enzymatic activity of these histyocites, parallel with a further increase in the ferric iron content of the histiocytes, as revealed by the large numbers of granules that gave a positive result with the Prussian blue test. As far as the argentophil reticulum was concerned, this group did not differ significantly from Group II.

Also in Group III, the lining cells of the sinus did not show any enzymatic activity or the presence of any iron-salt granules.

Discussion

The Prussian blue test, carried out with the precautions suggested by LISON, is a sensitive, specific and reliable test for the demonstration of ferric iron; it indicates the accumulation of haemosiderin and of ferritin in the histiocytic cytoplasm of the animals subjected to pinealectomy. It is difficult to explain the mechanism of this deposition in our experiments (Group II and III); in theory, a number of explanations may be considered.

The hypothesis of an *enhanced intestinal absorption* explains neither the hypertrophy of the argentophil reticulum nor the increase in the iron pigments after administration of trypan blue.

Pinealectomy may cause a decrease in the blood iron level, as the iron is transferred from the blood to the tissues.

Also this hypothesis is unable to explain all the splenic modifications that we have encountered.

A third possibility is that of *the freeing of the diencephalo-hypophyseal system, of the hypophysis or of the target organs* (thyroid, ovary, adrenal) as the consequence of the pinealectomy. There have been a very large number of publications on this subject, but very often these contradict one another, as has been shown in the monograph by CHIRICO and FERRATA.

Thus in the literature we find no unequivocal data to explain the iron deposition in the splenic histiocytes as a phenomenon due indirectly to the pinealectomy.

Consequently, we may consider the possibility that this phenomenon *is a direct consequence of the pinealectomy*. If the pinealectomy leads directly to a stimulation of the splenic portion of the RES, this might explain the iron deposition

as well as the hypertrophy of the reticulum and also the greater enzymatic activities as compared with the control animals. Also, the relationship between an accumulation of iron in the histiocytes and the enzymatic activity can be demonstrated with the aid of injections of trypan blue which bring about an increase in the enzymatic activities as well as a more pronounced iron deposition.

It would appear that for the moment this concept is a good working hypothesis, and we intend in due course to attempt to verify it.

Summary

After pinealectomy we were able to observe an accumulation of iron pigment in the histiocytes of the cords and of the follicles of the spleen in the rat, parallel with a slight increase in the enzymatic activity of the histiocytes and hypertrophy of the argentophil reticulum. The accumulation of iron and the enzymatic activity can be further increased by subcutaneous injection of trypan blue.

The working hypothesis is proposed that pinealectomy leads directly to an increase in the histiocytic activity in general and in the red cell destruction by the spleen in particular.

Über eine mögliche Beziehung zwischen der Glandula pinealis und dem Abbau von Erythrocyten in der Milz

Zusammenfassung

Nach Pinealektomie konnte eine Anhäufung von Eisenpigment in den Histiocyten der Pulpastränge und der Follikel in der Rattenmilz festgestellt werden; parallel damit ging eine leichte Erhöhung der enzymatischen Aktivität der Histiocyten und eine Hypertrophie des argyrophilen Reticulums. Die Anhäufung von Eisenpigment und Erhöhung der enzymatischen Aktivität kann weiter gesteigert werden durch subcutane Injektion von Trypan-Blau. Folgende Arbeitshypothese wird zur Erklärung dieser Tatsache vorgeschlagen: Die Pinealektomie führt unmittelbar zu einer Erhöhung der Aktivität der Histiocyten im allgemeinen und des Abbaues der roten Blutkörperchen durch die Milz im besonderen.

References

Balogh, K., and R. B. Cohen: Histochemical localization of oxidative enzyme systems in the human anterior pituitary. Endocrinology **70**, 874—879 (1962).

Burstone, M. S.: (1) Histochemical comparison of naphtol AS-phosphates for the demonstration of phosphatases. J. nat. Cancer Inst. **20**, 601—615 (1958).

— (2) Histochemical demonstration of acid phosphatases with naphtol AS-phosphates. J. nat. Cancer Inst. **21**, 523—539 (1958).

Chirico, G., e A. Ferrata: La regolazione endocrina dell'emopoiesi nella fisiologia e nella clinica, p. 11—103. Pavia: Haematologica 1958.

Lison, L.: Histochimie et cytochimie animales, 3rd ed., vol. 2, p. 690 et 777. Paris: Gauthiers-Villars 1960.

Pozzi, F., e G. Barbolini: Ricerche istoenzimologiche sugli istiociti della milza e del fegato di ratto con particolare riferimento agli istiociti in posizione endoteliale. Arch. de Vecchi Anat. pat. **45**, 523—557 (1965).

Thieblot, L., et H. Le Bars: La glande pinéale ou épiphyse, 1rst ed., p. 56—58. Paris: Maloine 1955.

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