NOTE
The Effect of a Spleen Dialysate on the Enzymatic Pattern of the Rodent Ovary*

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Synopsis

The stimulating effect of a spleen dialysate on the testicles and the accessory genital organs motivated the authors to test this dialysate on the ovaries. Also on the ovaries a different influence was observed.

In comparison to the control animals, the enzyme pattern: lactate dehydrogenase, steroid-3β-ol-dehydrogenase, alkaline phosphatase, succinate dehydrogenase, and glucose-6-phosphate-dehydrogenase is significantly changed. The effect is manifested by an increase of the tertiary follicles, increased follicular atresia and disquamating granulosa. On the basis of these findings we assume that the spleen dialysate is able to increase the gonadotropin effect on the ovaries.

In his monography about splenology, L. Arvy writes in 1965 that a direct correlation between the gonad function and the spleen still could not be proved. In examples of sexual dimorphism of the spleen, he was able to show that in many animal species the relative spleen weight in female animals is increased by at least 30–40% compared with the spleen least weight of the male animals. Annufrijew (1910) had already proved that the spleen in pregnant mice was bigger and heavier than in non-pregnant ones. In young mice, the oestrus obviously begins earlier after splenectomy (Radossavlevitch and Kostitch, 1929), whereas in mature rats with normal cycle, splenectomy leads to a prolongation of the cycles (Del Castillo, 1928). From human pathology we know of a genital insufficiency in women combined with a splenomegaly which disappears after splenectomy. In 1955 Lauda continued with a detailed investigation of such cases. He traced them back to a fibroadenia of the enlarged spleen.

Working for several years with a low-molecular, protein-free dialysate from calves' spleen showed a strong effect of spleen dialysate on the activity of the Leydig cell complexes and accessory genital organs in animals with intact hypophysis (Grigoriadis et al., 1969; Goslar et al., 1969; Breitenecker et al., 1972). This effect proved to be traceable both histologically and enzym-histochemically (Breitenecker et al., and Lunglmayr, 1972) in adult rats having an artificial unilateral kryptorchism. The enhanced activity of the Leydig cell complexes after injection of the spleen dialysate (SOLCOSPLEN®) is in parallelism with a high statistically proven increase of the plasma testosterone level (Breitenecker et al., 1972).

With the aid of the phallograph, Jovanovic (1972) was able to prove a stimulating effect of the spleen dialysate on potency disturbances both in men with organic troubles of the genitals and in frigid women by the aid of the clitorograph. Therefore we found ourselves confronted with the question what effects the spleen dialysate would give in animal experiments on the ovaries.

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Materials and Methods

For 14 days, 0.25 ml/100 g body weight of a spleen dialysate was given daily to 20 female infantile albino guinea pigs (body weight about 160 g) by i.m. injection. In the same way 20 control animals received 0.25 ml/100 g body weight of a physiological NaCl solution. Subsequently the animals were sacrificed by decapitation. The ovaries were removed immediately and one of them, frozen in dry ice for the enzyme histochemical investigations, the other one fixed in Bouin's solution and embedded in paraffine in the usual way.

I. Histological staining (paraffine sections 5 μ)
   Azane staining (Romeis, 1968, case: §1530).

II. Enzym-histochemical reactions (cryostate sections 16 μ)
   1) lactate dehydrogenase (LDH)
      acceptor: TNBT (s.Pearse, 1972).
   2) succinate dehydrogenase (SDH)
      acceptor: TNBT (s.Pearse, 1972)
   3) glucose-6-phosphate-dehydrogenase
      acceptor: TNBT (s.Pearse, 1972)
   4) steroid-3β-ol-dehydrogenase
      acceptor: TNBT (s.Pearse, 1972)
      (s.Wattenberg, modified by Levy et al., 1952)
   5) alkaline phosphatase
      pre-fixation 10 min in buffered formaline (s.
      Goslar and Bock, 1970) at 4°C. Azo-coupling
      method (Barka and Anderson, 1963).

Results

Azan-staining

In the control animals many primary and secondary follicles can be observed. The tertiary follicles are beginning to develop (Fig. 1). In the test animals, a substantial increase in the formation of tertiary follicles is obvious. The cavum folliculi is very large. The granulosa is decreasing almost everywhere. Detached granulosa cells appear in the cavum folliculi. The theca organs are highly developed (Fig. 2).

The reaction of lactate-dehydrogenase shows the granulosa of the forming tertiary follicles to be only slightly active in the control picture (Fig. 3) Theca interna having a highly positive reaction. The theca organs also appear strongly positive. In the test animals, the disquamatig granulosa seems very active in the heavily enlarged tertiary follicles as already described by Zerbian (1966) after application of large amounts of oestrogen. The theca interna is enlarged and histochemically strongly active, the same behaviour can be observed in the enlarged and numerous theca organs in the surroundings (Fig. 4).

Whereas, with the succinate dehydrogenase, the granulosa appears only very slightly active and the theca interna with exception of the theca organs does not show a strong reaction after application of spleen dialysate (Fig. 5). We find a considerably higher activity of this enzyme in the theca organs (Fig. 6). The reaction of the two described enzymes seems to be about equal. The steroid-3β-ol-dehydrogenase shows only a week increase of activity in the theca organs. The glucose-6-phosphate dehydrogenase is clearly increased in the disquamatig granulosa after treatment with spleen extract (Fig. 7). In the control animals, the alkaline phosphatase appears very active in the theca interna and the theca organs (Fig. 8) in the best animals (Fig. 9) obviously very much higher.

Discussion

Considering the above described findings, at first the great increase and enlargement of the tertiary follicles attracts initial attention. Moreover, in the enzyme picture the theca organs and their cells appear greatly activated, when compared with the controls.

In the granulosa membrane SDH and glucose-6-phosphate-dehydrogenase are clearly more positive, and, on the other hand, disquamatig and dissolution processes of the granulosa are to be seen.

This proves a stimulation of the follicle maturation, and, on the other side, an activa-
tion of the cells producing oestrogen. In order to interpret the results, we refer to the effect of the spleen dialysate on male animals. After application of the spleen dialysate, the Leydig cell complexes were considerably enlarged, the enzyme activities (especially the steroid-3β-ol-dehydrogenase) raised, the spermiogenesis was activated and the plasma-testosterone significantly increased (Grigoriadis et al., 1969; Goslar et al., 1969; Breitenecker and Lunglmayr, 1972; Breitenecker et al., 1972).

The analogous function of Leydig cell complexes with theca organs, respectively internal theca cells, consequently suggests (according to the morphological and enzyme-histochemical picture) also an increase of the oestrogen level on female animals, as the testosterone of male animals raises under the application of the spleen dialysate. This hypothesis is supported by the increased disquamation of the granulosa after high oestrogen doses (see Zerbian, 1966). We shall soon report about plasma oestradiol determinations by means of radioimmuno-assay, in order to confirm these assumptions.

The present results prove that the spleen dialysate we used has not only a stimulating effect on male, but also on female gonades. It remains to be clarified by further trials now in progress, whether the hypophysis is induced to produce more gonadotropin or whether a direct effect on the gonades (perhaps in the sense of an increased accessibility to gonadotropin) is exerted.
References