THE ACTION OF SULPHANILAMIDE ON RABBITS' LENSES IN VITRO*†

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Introduction

IN a communication published some years ago¹ I described a number of introductory experiments which had the object of showing that the perfusion culture method of de Haan¹⁴ was excellently serviceable for keeping alive rabbits' lenses *in vitro* for many days. This was the first time that it seemed possible to preserve that extraordinarily vulnerable tissue in such a good condition outside the body. All attempts made in that direction had always miscarried until then¹¹.

These preliminary experiments convincingly demonstrated the usefulness of this method. Of course good results can only be obtained when the lens is not injured during the operation.

One of the most striking advantages of the perfusion culture method is the *continual* renewal of the nutrient medium. This liquid, obtained from the abdominal cavity of rabbits and in its composition resembling aqueous humour very much, passes the explants in an uninterrupted current. Probably it is for that very reason that I succeeded in obtaining such good results.

The more important facts of my research work, showing evidence of the high vitality of the explanted lenses, may be summarized as follows:—

1. Unaltered metabolism even in explants several days old. The lenses maintain their normal transparency for many weeks.

2. Lenses of young animals show symptoms of growth, apparent from mitotic cell divisions in the equator region.

3. Ascorbic acid can freely diffuse through the capsule in both directions. When the nutrient liquid contains an adequate quantity of this vitamin its concentration in the lens has normal values too². This indicates that the permeability of the capsule of the explanted lenses does not change.

4. Regeneration of experimentally wounded capsules starts and goes on *in vitro* in exactly the same way as *in vivo*. The final result is in both circumstances a newly formed capsule³.

In subsequent experiments not only the lens but also other ocular tissues, such as the iris, the choroid, the retina and the ciliary body were explanted with equally good results⁴. This enlargement of the

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usefulness of our culture method enabled us to investigate the influence of radiant energy on the crystalline lens, covered or not by the iris⁵. The conception of Vogt and co-workers¹⁵ that the lens absorbs the light rays of long wave-lengths in large quantities could be refuted. My experiments showed that the explanted lens only became cataractous under the influence of heat rays, when it was covered by the pigmented iris. Thus my experiments agreed fully with those of Goldmann¹³. Although Goldmann and I worked along quite different lines, we both came to the conclusion that the lens is *indirectly* burnt by the heat rays absorbed in the pigmented iris.

This short enumeration of the results obtained with the explanted rabbits' lenses may give an impression of the possibilities of this culture method.

Subsequently I carried out further investigations with the object of determining whether this method could be made subservient to testing drugs on their toxicity. I chose sulphanilamide (prontosil album), as this drug is easily soluble in water and as it was used in our laboratory to fight against probable infections of the nutrient liquid.

There was still another reason to investigate the influence of sulphanilamide on the lens, as it is known that this drug inhibits the enzyme action of carbonic anhydrase. This enzyme (discovered by Brinkman and Margaria¹²) catalyzes the reaction $\text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}$. Now the lens contains a very high concentration of this enzyme and as I could show⁶ that in cataractous lenses the concentration of carbonic anhydrase decreases considerably, it is probable that an intimate relation exists between this enzyme and the transparency of the lens. In other words the behaviour of the explanted lens under the influence of sulphanilamide can enable us to get an impression of the toxic action of this drug.

A simple method to determine the concentration of carbonic anhydrase in lenses (and in other ocular tissues) is described in a preceding article⁷. Two later papers deal with some activators and inhibitors of this enzyme^{8, 9}.

For the details of this culture method and the technique I must refer to my former publications.

At the end of each experiment the enzyme concentration and the content of sulphanilamide in the lenses is determined. Sometimes the lens is microscopically examined, this, however, does not yield any important data.

Experimental results

Introductory experiments taught us that a concentration of 5 mgr. per cent. of sulphanilamide in the nutrient fluid (a concentration lower than the blood concentration in the usual therapeutic dose)

was quite harmless for lenses of adult rabbits. In eight day old explants the lenses did not show any opacities (perfusion velocity of the nutrient fluid 1 drop in about 50 to 60 seconds).

A content of 20 mgr. per cent. of sulphanilamide was not noxious to lenses of a seven week old rabbit. After a stay of three days in the culture vessel the lenses were still quite clear.

When, however, the concentration was raised to 400 mgr. per cent. the lenses of a four week old rabbit got very opaque in the short time of two hours. After 15 hours transparency was so much impaired that letters on an underlying paper could no longer be read. After three days the culture was stopped. The opacities were especially localized in the posterior cortex, but also the anterior cortex showed some of them. The lenses contained 385 mgr. per cent. of sulphanilamide, demonstrating that the capsule is freely permeable to this drug. The enzyme action was reduced to 1/5 of its original value.

Though the deleterious influence to lenses of very young animals was evident, the toxicity of sulphanilamide for lenses of adult rabbits was less pronounced. It was not until after five days that lenses of the latter in the same concentration (400 mgr. per cent.) showed some opacities. After eight days the enzyme action in these lenses was reduced to less than 1/6 of its original value.

Conclusions

The perfusion culture method proved to be able to give reliable information on the toxic action of chemicals. With the aid of the same method too, I was formerly able to give an affirmative answer to the question whether galactose has a *direct* noxious influence on the lens¹⁰.

The present experiments have shown that sulphanilamide has not any injurious effect on rabbits' lenses when the concentration is not raised above the level reached in normal therapeutic doses. Yet, some caution is advisable in using this drug. Mrs. Schappert-Kimmijser told me (verbal communication) that in a 43 year old patient, suffering from erysipelas faciei and treated with prontosil for no less than 4 months, cataractous changes in both lenses of capsular and sub-capsular character developed.

There was a striking difference between the behaviour of young and old lenses in my experiments. The younger the animals were the more their lenses were susceptible to the noxious influence of sulphanilamide

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A CASE OF CONGENITAL VERTICAL SHORTNESS OF THE LIDS COMBINED WITH TETRASTICHIASIS*

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CONGENITAL shortness of the lids was extensively studied by E. Fuchs^{1,2} in 1885. Congenital absence (ablepharon) or maximal shortness (microblepharon) of the lids has been observed repeatedly, partly in monsters, and also in otherwise normal individuals (cf. Manz, *Handbuch. d. Ophthalm.*, *Graefe-Saemisch.*, Vol. II, p. 103). Stellwag described mainly the smallness of the lids in the horizontal diameter and the ensuing shortness of the palpebral fissure in this direction but not what, in the opinion of the writer, should be called vertical shortness of the lids. It was left to Fuchs to coin the expressions "height of the lid" and "vertical extension of the skin of the lid". "Height of the lid" is the maximum distance between the lid margin and the centre (i.e. half the breadth) of the eyebrow. In measuring this height the individual is asked to close the eyes

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