CONSTITUTIONAL DIFFERENCE IN LIPID CONTENT OF ADRENALS IN TWO STRAINS OF MICE AND THEIR HYBRIDS

By

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This report is part of a study of the possible association between certain constitutional traits and susceptibility to leukemia in the Ak strain of mice. This strain, or at least the substrain in our laboratory in Oslo, is characterised by a reduction of the adrenocortical lipid concentration as well as by hyperplasia and delayed physiological involution of the thymus. Law & Speirs (1947) and Law (1947) have demonstrated that the leukemic process is influenced by the adrenal cortex. Furth (1946) showed that the incidence of leukemia in the Ak strain was reduced after thymectomy. It was therefore considered justifiable to study the «adreno-thymic» constitution of the Ak strain in order to test the hypothesis of an association between constitution and susceptibility to leukemia. A preliminary report of this investigation has been published (Arnesen, 1953).

The adrenal cortex of various animals has been the subject of extensive chemical and histochemical studies which aimed at clarifying the functional state of the organ. There are several histochemical methods for the study of tissue lipids, but none of these methods are sufficiently specific to give exact information about the chemical constitution of the compounds studied. It appears to be generally accepted, however, that a battery of positive reactions including sudanophilia, phenylhydrazine reaction, Schiff reaction, semicarbazide reaction, Reichstein's ammoniacal silver reaction, the Schultz (Liebemann-Burchardt) reaction, birefringence, autofluorescence and acetone solubility are indicative of ketosteroids. The individual reactions are not specific, but keto-

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steroids are the only known substances which react positively with all these tests (Greep & Deane, 1949).

Whitehead (1933) and Vicari (1943) have studied the variations in the distribution of sudanophilic lipids in the adrenal cortex of mice. Vicari and Vicari & Little (1945) compared various pure strains and found an inverse relationship between the amount of cortical lipids and incidence of mammary tumour, but gave no definite proof of a biological association.

Levin (1948) has studied »the possible relationship between adrenocortical function and the leukemic state« in the Ak strain. He made chemical determinations of the adrenal cholesterol, and found that the development of spontaneous or transplanted leukemia was associated with a decrease in the concentration of adrenal cholesterol.

MATERIAL AND METHODS

The Ak mice used in this study have been kept in our laboratory since 1950, and have given an incidence of spontaneous lymphoid leukemia of almost 90 per cent. The disease occurs from the age of about 25 weeks onwards. For comparative investigation as well as in hybridization experiments the »White label« (WLO) strain (Kreyberg, 1952) has been used. In this strain no case of spontaneous leukemia has been observed. Both strains are strictly inbred by brother-sister matings. Reciprocal crosses have been made, and a considerable number of F₁ and F₂ animals as well as back-crosses to both parental strains have been raised. The animals used in this study were sacrificed when 12 weeks old (84–90 days) and were in good health. A few animals were examined at 11, 22, 30 days of age. In addition the following experiments were done:

A group of males were castrated at the age of 21 days and the adrenals examined at 12 weeks. Several groups of mice at different ages were treated with Corticotrophin (ACTH) prepared by the firm Nyegaard & Co. A/S, Oslo and standardized according to Sayers & Sayers’ ascorbic acid test. This compound was given in doses of 1 I.U. twice a day for three days, and the experimental and control animals were sacrificed at various time intervals after the last injection.

The animals were killed by fracture of the cervical spine, and the adrenals taken out immediately and fixed in neutral 10 per cent formalin. Frozen sections were prepared after gelatin embedding, cut at 10–15 µ and routinely treated with Sudan III in 70 per cent alcohol. Untreated sections were mounted for examination in polarized light. In some instances the Schultz cholesterol test was applied. The Sudan treated sections were microphotographed through a green filter at 40 times enlargement.

In this report only the concentration of histochemically demonstrable lipids in the adrenal cortex is considered.

RESULTS

There is no marked strain difference in the size of the adrenals, but weight determinations have not been made on a large scale. In both strains the male glands are smaller than the female glands.
A. The WLO strain presents adrenals corresponding to those of other strains examined, but not included in this report. Both males and females have adrenals with a definite yellow colour, the male glands being paler. Figs. 1 and 2 show the microscopical appearance of the Sudan stained sections of WLO male and female adrenal glands. There is a uniform, rich concentration of lipids throughout the whole permanent cortex. In the female gland the degenerating x-zone is conspicuous (Brown degeneration).

B. The Ak strain. The adrenals differ macroscopically from those of the control strain. The Ak male adrenals have a deep red colour, and the female glands are pale with a faint yellowish tint. Fig. 3 shows the adrenal of an Ak male. There is a narrow rim of lipids in the zona glomerulosa just underneath the capsule. The rest of the cortex is practically free from sudanophilic lipids. The picture of the Ak female adrenal is shown in Fig. 4. The concentration of lipids is higher than in the Ak male gland, but much reduced as compared with the WLO female. The degenerating x-zone is clearly seen even in the Ak female gland.

The differences demonstrated by the Sudan technique are also revealed by the Schultz test and by examination in polarized light.

C. The hybrids. In the hybrids the adrenals are referred either to the Ak or WLO type. Usually the adrenal type can be determined by the microscopical appearance of the gland. In most cases microscopical determination have also been carried out. Table 1 presents the distribution of the adrenal type in the F1, F2, F1 × Ak and F1 × WLO hybrids. There does not appear to be any influence that can be attributed to sex or reciprocity of mating. In the F1 generation all animals have adrenals of the WLO type. In F2 there is a ratio WLO: Ak very close to 3:1, and in F1 × Ak the ratio approaches 1:1, whereas all F1 × WLO animals have WLO adrenals.

D. The adrenocortical lipids before puberty. The reduction of lipid concentration is not demonstrable in infant animals, but only occurs after sexual maturity has been reached. Figs. 5, 6 and 7 demonstrate the adrenal cortex of Ak male mice at 11, 22 and 30 days of age. At 11 and 22 days the picture of lipid concentration corresponds to that of WLO animals of the same sex and

<table>
<thead>
<tr>
<th>Ak adrenal type</th>
<th>WLO adrenal type</th>
<th>Total number</th>
</tr>
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<tbody>
<tr>
<td>F1</td>
<td>0 ( 0 %)</td>
<td>90 (100 %)</td>
</tr>
<tr>
<td>F2</td>
<td>61 (27 %)</td>
<td>165 (73 %)</td>
</tr>
<tr>
<td>F1 × Ak</td>
<td>169 (48 %)</td>
<td>183 (52 %)</td>
</tr>
<tr>
<td>F1 × WLO</td>
<td>0 ( 0 %)</td>
<td>184 (100 %)</td>
</tr>
</tbody>
</table>

Table 1.
Adrenal type in Ak – WLO hybrids.

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Fig. 1: WLO ♂, 12 weeks old. Fig. 2: WLO ♀, 12 weeks old. Fig. 3: Ak ♂, 12 weeks old. Fig. 4: Ak ♀, 12 weeks old. Fig. 5: Ak ♂, 11 days old. Fig. 6: Ak ♂, 22 days old. Fig. 7: Ak ♂, 30 days old. Fig. 8: Ak ♂, castrated 21 days old, killed at 12 weeks. Fig. 9: Ak ♂, 15 weeks old. Treated with 6 I.U. ACTH, killed 9½ hours after the last injection. Fig. 10: Ak ♂, ACTH control.
age. The 30 day adrenal shows beginning lipid reduction, which is probably completed at about 40 days of age.

E. Effect of castration. In Fig. 8 the adrenal of an Ak male is shown which was castrated when 21 days old and killed at the age of 12 weeks. It is seen that the outer zone of the cortex is fairly well filled with sudanophilic lipids, but the concentration is neither so regular nor so high as in the WLO male. It is also to be noted that a broad inner portion of the cortex does not contain lipids.

F. Effect of ACTH treatment. Ak males treated with 6 I. U. ACTH during 3 days and killed $9/2$ hours after the last injection, present evidence of a marked storage of sudanophilic lipids in the outer $2/3$ of the adrenal cortex (Figs. 9 and 10).

CONCLUSIONS AND DISCUSSION

In the Ak strain a spontaneous, permanent lipid depletion of the adrenal cortex is regularly found, as demonstrated by the Sudan technique. When considered as a qualitative variable, this state of depletion appears to depend on a single, recessive Mendelian factor, and may be regarded as a constitutional anomaly. The process is in some way or other influenced by the sexual glands, as demonstrated by the findings in prepubertal or castrated adult animals. This has only been clearly demonstrated in males.

It is not intended to discuss in detail the physiological significance of the histomorphological picture of spontaneous, permanent lipid depletion of the adrenal cortex. It may be assumed, however, that the degree of sudanophilia supported by determinations of anisotropic and Schultz positive material gives an indication of the synthetic and secretory activity of biologically active steroids.

The adrenals demonstrated in this paper (Figs. 1–10) are at rest. Even if they may be interpreted on a functional basis, it is not a priori clear whether the Ak adrenals are hyper- or hypofunctional. A high level of synthetic activity combined with a highly increased rate of secretion would give a static picture of lipid depletion. Reduced synthesis combined with normal or increased secretion would also tend to give an "empty" cortex. Variations in weight would help in solving the problem of hyperfunction or hypofunction. In the case of the Ak adrenals no gross divergence in size from the control strain has been observed. At the present time the only clue to the determination of the functional state of the Ak adrenal is the concomitant hyperplasia and delayed involution of the thymus. This fact supports the hypothesis of at any rate a partial hypofunction of the adrenal glands or the pituitary-adrenocortical system.

When considering possible causes of the anomaly described here, one might
assume a primary defect in the adrenal glands themselves, affecting the capacity to produce and/or store sudanophilic lipids. On the other hand, there might be a disturbance in the adrenocorticotrophic activity of the hypophysis resulting in secondary changes in the adrenals. The ACTH experiments give some information on this point. It appears that the Ak strain adrenal is able to produce and store sudanophilic lipids under hypophyseal stimulation. The doses of ACTH necessary to obtain a clear cut effect are, however, high, and a quantitative difference in the reactivity to adrenocorticotrophin as compared with the adrenals of »normal« mice cannot be ruled out.

The problem of the association between the »adreno-thymic« constitution and susceptibility to leukemia in the Ak strain, referred to in the introduction, will not be discussed here.

**SUMMARY**

The author describes the differential lipid content of the adrenal cortex in the Ak and WLO strains of mice. The Ak adrenals present a spontaneous permanent lipid depletion depending on a single, recessive Mendelian factor and also upon the presence of mature sexual glands. The lipid content of the Ak adrenal cortex can be restored by treatment with high doses of ACTH. The interpretation of the experimental findings is briefly discussed.

**REFERENCES**

Levin, L.: Cancer 1, 413, 1948.