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Changes of Vitamin A Distribution in Choline Deficiency.

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Choline-deficient diets produce fatty livers (Best[1]). Griffith[2] demonstrated that in addition to the liver changes, young rats develop an acute syndrome with hemorrhagic renal degeneration, splenic enlargement, ocular hemorrhage and thymic involution. Livers of experimental animals made fatty by diets[3] or toxic agents[4] ordinarily are rich in vitamin A which can be seen histologically in the fat droplets.[5] Choline feeding stimulates the removal of vitamin A as well as fat from the liver.[3] It seemed important, therefore, to determine whether a choline deficiency reveals a similar dependence of the vitamin A storage upon fat deposition in the liver. Since human fatty livers may be either rich or poor in vitamin A,[6] this question assumes clinical significance.

Thirty-two rats, 21 to 28 days old, were fed a low choline, low methionine, high cystine diet,[7] supplemented with carotene and the crystalline vitamins.‡ Control animals received 25 mg choline chloride daily. Livers, kidneys and adrenals were examined by routine histologic procedures and for vitamin A under the fluorescence microscope.[6] The vitamin A of some livers was determined chemically.§

Fourteen rats on the deficient diet showed fatty livers of varying

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1 Best, C. H., Hershey, J. M., and Huntsman, M. E., *Am. J. Physiol.*, 1932, **101**, 7.

2 Griffith, W. H., *J. Nutrition*, 1941, **22**, 239.

3 Thorbjarnarson, T., and Drummond, J. C., *Biochem. J.*, 1938, **32**, 5.

4 Lasch, F., *Klin. Wchnschr.*, 1935, **14**, 1070.

5 Popper, H., and Steigmann, F., in preparation.

6 Popper, H., *Arch. Path.*, 1941, **31**, 766.

7 Engel, R. W., and Salomon, W. D., *J. Nutrition*, 1941, **22**, 109.

‡ Generously supplied by Merck and Co., Inc., Rahway, N.J.

§ We thank S. S. Zevin for these determinations (Method of Josephs, H. W., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 112.

degrees within 2 to 10 days. The vitamin A fluorescence at first increased over the low levels in the newly weaned animals. After the third day, however, the fluorescence diminished steadily as the liver fat accumulated. Only in irregular patches, did Kupffer cells and large fat droplets of surrounding liver cells show appreciable vitamin A fluorescence. This fluorescence diminished towards the periphery of the patches. Outside of these areas, the fluorescence was completely absent. Shortly before death of the rats, such patches were very small and the Kupffer cells free from vitamin A. Thus, the total vitamin A in the liver at this time, was much less than at the start of the experiment, despite liberal carotene supplements. Livers of control animals receiving choline daily were rich in vitamin A. About the sixth day, the rats showed the characteristic hemorrhagic renal changes.[8] Marked vitamin A fluorescence appeared in the interstitium of the renal cortex in a distribution following the nephron. The adrenals were hemorrhagic in some animals, vitamin A-free in all. Ocular hemorrhage was occasionally seen in rats displaying the most severe changes. No sex difference in vitamin A distribution was apparent.

Ten rats which either spontaneously had survived the critical period of choline deficiency (10 days) or had received subnormal choline supplements were sacrificed after 20 to 90 days on the diet. Their livers were fatty with no vitamin A in Kupffer cells and only patchy traces or none at all in fat droplets. By chemical analysis, from 1.3 to 19 International Units of vitamin A per gram were found, in contrast to the normal value of over 200 I.U. Male animals showed much less vitamin A than females both chemically and histologically. The kidneys seemed normal upon routine histological examination except for scars indicating some past injury. With the fluorescence microscope, however, the cortical interstitium appeared extremely rich in vitamin A in a nephron-bound distribution.

The progeny of females on the deficient diet showed fatty livers with little vitamin A. This condition was accentuated if mother and litter were further maintained on the choline-free ration. Liver vitamin A values as low as 0.5 I.U. per gram were observed.

Liver storage of vitamin A thus depends not only upon dietary ingestion and fat deposition, but also upon choline intake. The disappearance of vitamin A from the fatty livers of choline-deficient rats is not related to the acute stage with "hemorrhagic degeneration" since it is even more marked in the chronic experiments. Nor is inability of the liver to convert carotene into vitamin A a major

[8] György, P., and Goldblatt, H., *J. Exp. Med.*, 1940, **72**, 1.

factor, since preliminary experiments replacing carotene by vitamin A in the diet reveal the same picture. The excessive vitamin A of the kidneys, never seen normally,[9] excludes an absorption difficulty. Furthermore, the wealth of vitamin A in histologically normal as well as hemorrhagic kidneys would seem to exclude renal pathology as a fundamental cause. Whether the vitamin A deposits in the kidney are due to compensatory storage or urinary excretion cannot yet be answered.

Summary. Rats on a choline-poor diet containing liberal supplements of carotene develop fatty livers poor in vitamin A. The kidneys of these animals are extremely rich in vitamin A.

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Localized Maturation of Lid-closure Reflex Mechanism by Thyroid Implants into Tadpole Hindbrain.*

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The lid-closure (wink) reflex of amphibians—retraction of the eye bulb upon tactile stimulation of the cornea or surrounding skin—appears comparatively late in development. Urodeles display it first in advanced larval stages, anurans at or about the time of metamorphic climax. In the latter group its dependence upon the metamorphosing agents was demonstrated by experiments in which metamorphosis was either suppressed (hypophysectomy) or advanced (hyperthyroidism);[1] in the former case, the reflex failed to appear unless thyroid was administered, while in the latter case its onset was advanced almost, though not fully, as much as was the transformation of the body. Both the sensory (trigeminal)and motor (abducens) components of the reflex develop full functional capacity in very early larval stages, but their central linkage into a workable reflex mechanism is not effected until at the time of metamorphosis.

The close association with metamorphosis suggested that maturation of the central mechanism of the reflex might be under direct

9 Popper, H., and Greenberg, R., *Arch. Path.*, 1941, **32**, 11.

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1 Kollros, J. J., *J. Exp. Zool.*, 1942, **89**, 37.