medium were undoubtedly much less extensive. Nevertheless, the mere aging of sterile medium changed it in such a way that it acquired the property of accelerating growth of *C. campylum* when added to equal or larger volumes of fresh peptone solution.

These observations have a bearing upon the question of "biologically conditioned" medium, and upon the "allelocatalytic effect" described by Robertson and others. The results obtained in Series I, III and VI might reasonably be attributed to a "biological conditioning" brought about during growth of the ciliates, if it were not for the fact that aged sterile medium produced comparable effects. Our findings do not demonstrate that the factors producing acceleration of growth are identical in old culture filtrates and in aged sterile medium, and it is possible that they are not the same. On the other hand, it is equally obvious that a "biological conditioning" cannot be invoked as the sole explanation for the accelerating effects of old culture filtrates.

Our results show further that, for studies on the "allelocatalytic effect" in ciliates, old culture fluid transferred to experimental cultures may accelerate growth in proportion to the amount of fluid carried over. Hence, in such investigations on growth in relation to initial density of population, it is imperative that the organisms in the inocula should be washed thoroughly in order to remove old culture fluid. Failure to observe such a precaution may well be responsible, at least in part, for some of the apparent conflicts in current views regarding the "allelocatalytic effect".

11120

Histological Demonstration of Vitamin A in Rats by Means of Fluorescence Microscopy.

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Vitamin A reveals a green fluorescence in ultraviolet light which disappears rapidly during irradiation.[1] This was observed under the fluorescence microscope using dilute aqueous emulsions of vitamin A concentrates. The striking green of the droplets fades during the

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1 Peacock, P. R., *Lancet*, 1926, **2**, 328.

observation. The same behavior is noted in some animal tissues and may be attributed to the presence of vitamin A. Querner,[2] describing inclusions in epithelial cells of liver and adrenal, and Jancso[3] demonstrating fluorescence of the pigment epithelium of the eye, made this assumption. The assumption that such a fluorescence is due to vitamin A can be supported now by the following facts. We found this fading fluorescence always located in lipoids of the body and absent after treatment of the tissue with alcohol or acetone. Its distribution in the body agrees with that of vitamin A as determined chemically. Animal experiments, as those reported later and a few briefly mentioned by Querner offer evidence that this particular fluorescence is due to the presence of vitamin A.

Thin pieces of tissue were fixed in excess of 10% formalin and frozen sections made within 24 hours after fixation and examined under the fluorescence microscope. Inadequate and prolonged fixation must be avoided because the fatty bodies acquire a disturbing bluish fluorescence due to oxidation.

The observation of the spontaneous fluorescence of vitamin A could be supported by staining with fluorescing dyes (fluorochromy). Fats give a bluish fluorescence with methylene blue which in presence of vitamin A is surpassed by the fading green fluorescence. By changing the ultraviolet to a ground glass filter one can observe the blue stained slide in visible light and exactly localize the fluorescing inclusions. In addition, the carrier substances (lipoids) can be demonstrated by fluorescing dyes.

Twenty-three rats were grouped for vitamin A assay by Ruven Greenberg† according to U.S.P. method XI (revised 1937) and checked chemically. In 8 positive controls a regular distribution of smaller and occasionally larger droplets in the parenchyma of the liver was observed which showed a striking fluorescence fading during irradiation. The cytoplasm also revealed a green fluorescence which faded to blue. In the Kupffer cells there were also aggregations of small droplets which manifested a green fluorescence. In 7 deficient rats no green fluorescence was demonstrated; only a faint bluish fluorescence of the cytoplasm as in positive controls after fading of the green fluorescence was observed.

Six deficient animals which received more than 6600 units of vita-

2 Querner, F. von, *Kli. Wo.*, 1935, **14**, 1213.

3 Jancso, N. von, and Jancso, H. von, *Biochem. Z.*, 1936, **287**, 289.

† Thanks are due to Ruven Greenberg for the preparation of the animals and the chemical determinations (method of Guilberts, H. R., and Hart, G. H., *J. Nutr.*, 1934, **8**, 25).

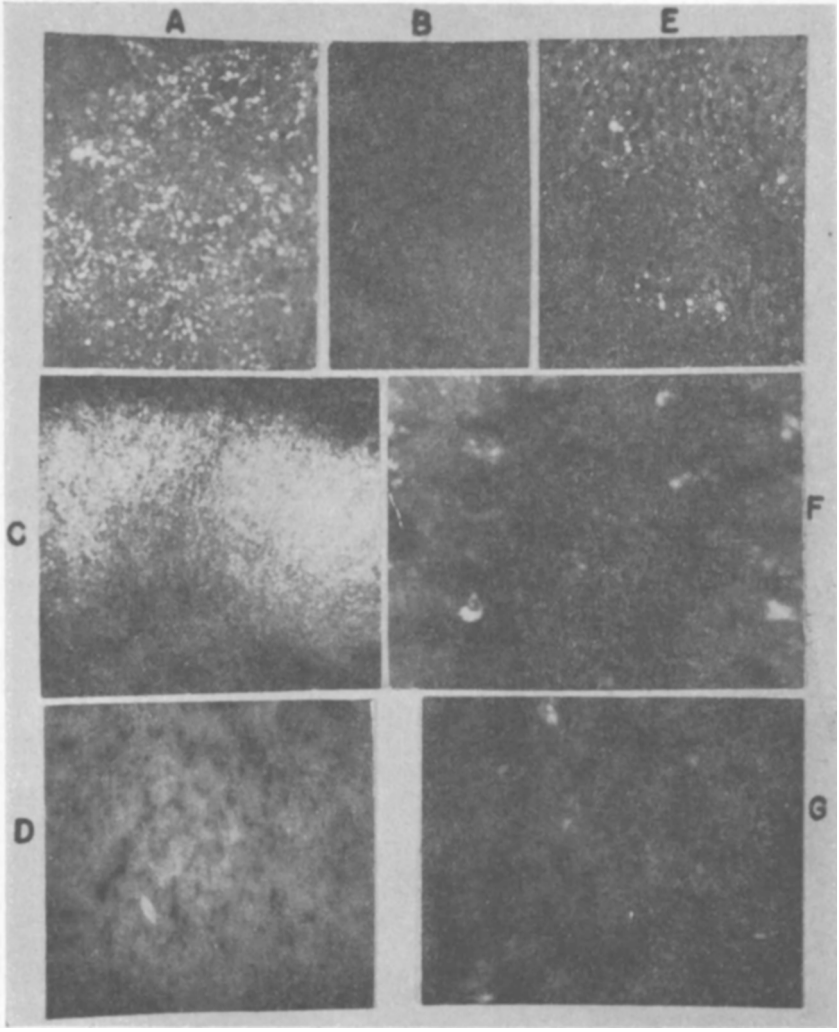

FIG. 1.

Fluorescent Microphotographs.

A. Liver of control rat. Green fading fluorescing droplets irregularly distributed in parenchyma and Kupffer cells.

B. Liver of vitamin A deficient rat.

C. Adrenal of control rat. Fine green fluorescing droplets in the cortex of the adrenal.

D. Adrenal of vitamin A deficient rat.

E. Human liver, low power. Fading green fluorescence in irregularly distributed fat droplets in liver cells, and in Kupffer cells.

F. Human liver, high power. Fading green fluorescence of Kupffer cells and of small acetone soluble droplets distributed along the edge of the epithelial cells.

G. Human liver, high power. Fading green fluorescence of Kupffer cells and the angular lipofuscin lumps which are in the center of the liver cords.

min A within sufficient time to allow storage showed many green fluorescing inclusions in epithelial and Kupffer cells, and chemically more than 1000 international units of vitamin A per gram of liver tissue. Two other animals which received 3300 and 6600 units within 3 hours before killing showed only very little green fluorescence.

The adrenals of positive controls and deficient animals after repletion revealed many small droplets with green fading fluorescence in the epithelial cells of the cortex, especially in the middle layer. In the deficient animals no such green fluorescence was present.

In the liver of 6 newborn rats there was no green fluorescence in the cytoplasm and only a few fluorescing droplets in epithelial and Kupffer cells. This is in agreement with chemical determinations of Ellison and Moore.[4] In the adrenals there was no green fluorescence.

In rats deficient in vitamin B_1 (2 rats), B_2 (2 rats), and D‡ (4 rats) normal amounts of fluorescing inclusions were found in liver and adrenal.

The liver of rabbits, monkeys, dogs, guinea pigs, mice, and frogs revealed essentially the same findings as the liver of normal rats.

11121

Production of Renin by Constricting Renal Artery of an Isolated Kidney Perfused with Blood.

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Experimental hypertension resulting from constricting the renal artery with a clamp (Goldblatt, Lynch, Hanzel and Summerville[1]) or as a result of perinephritis produced by cellophane or silk (Page[2]) is believed by many investigators to be of humoral origin and specifically to be caused by the liberation of renin from the kidneys. This renin may in turn interact with renin-activator (Kohlstaedt, Helmer

4 Ellison, J. B., and Moore, T., *Biochem. J.*, 1937, **31**, 165.

‡ For these animals I wish to thank Dr. H. J. Cannon, Director of the Laboratory of Vitamin Technology, Chicago.

1 Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

2 Page, I. H., *Science*, 1939, **89**, 273.