Uptake and Autoradiographic Demonstration of Vitamin A and/or its Metabolic Derivatives in Organ Cultures of Rat Prostate

The epithelia of the respiratory tract, salivary glands, cornea and genito-urinary tract do not normally exhibit the presence of vitamin A[1], although these epithelia are the first to undergo the squamous keratinizing metaplasia of vitamin A deficiency. This suggests that it may not be the vitamin itself that is essential for the epithelia, but some metabolic derivative; or alternatively, the amount of the vitamin in the cell is so minute, that it is not detectable by the usual fluorescent and chemical methods. In the present study, autoradiographic and chemical techniques were employed to demonstrate the uptake of vitamin A by rat prostate glands in organ culture.

Materials and methods. Ventral prostate glands from 80–100 g male rats were cut into small fragments and explanted as organ cultures in synthetic medium 199 in an atmosphere of air; the method was that described by LASNITZKI[2] and DINGLE et al.[3]. Each culture dish contained a total of approximately one mg of tissue fragments and 1.5 ml of culture medium.

(1) For the autoradiographic experiment the explants were permitted to become adjusted to the culture conditions for 24 h, the medium was then removed and replaced with 1.5 ml of fresh medium containing either 0.3 μg/ml or 3.0 μg/ml of C^{14}-retinyl acetate [vitamin A (carbinol-C^{14}) acetate, specific activity — 8.9 μc/mg, containing 0.5% w/w each of butylated hydroxyanisole and butylated hydroxytoluene as antioxidants, Radiochemical Centre, Amersham, England]. In addition, 0.5% albumin was added to stabilize the vitamin. Samples of the explants were removed from culture at intervals of 0.5, 1, 4, 24 and 48 h after the addition of the radioactive retinyl acetate, washed free of excess vitamin, and frozen immediately in isopentane immersed in liquid nitrogen. Autoradiographs of the explants were prepared by the cryostat-microtomy method of APPLETON[4] for the localization of soluble materials and were exposed in total darkness for 4 months at — 25 °C.

(2) In a parallel experiment, unlabelled retinol or retinyl acetate was added to the explants of prostate under identical experimental conditions to those described for the C^{14}-retinyl acetate. Samples of the explants (10–15 mg of pooled tissue for each analysis) were removed at the time intervals mentioned above, washed free of excess vitamin, 'homogenized' in a small volume of ethanol, the vitamin extracted from the tissue with a water-ethanol-petroleum ether (1:2:4) system and the extract chromatographed on partially deactivated alumina by a micro-modification of the method of CUNNINGHAM and MORTON[5]. The quantity of vitamin was determined with a Unicam S.P. 700 spectrophotometer at 328 nm using 4 cm light path cuvettes. The vitamin was then destroyed by UV-irradiation, a post-irradiation reading made and the difference in readings taken as the measure of the concentration of the vitamin.The vitamin A content of culture medium samples were also analyzed.

Results. (1) Figures 1 and 2 show the localization of labelled material in the prostate explants in the first (autoradiographic) experiment, at the dose level of 0.3 μg/ml of C^{14}-retinyl acetate. The grains appear over cytoplasmic and nuclear areas in both epithelia and connective tissue. The total number of grains present over the tissue increases from the 30 min to the 48 h time interval. A similar increase in tissue grain count over the 48 h period (not illustrated) was also observed at the 3.0 μg/ml dose level.

(2) The results of the second (analytical) experiment are shown in the Table. The data refer only to the 3.0 μg/ml dose level, since the method used was not sensitive enough to detect the vitamin at the lower dosage. It appears that the tissue takes up the vitamin within 30 min and maintains it at a constant level for at least 48 h;

[1] H. POPPER, Archs Path. *31*, 766 (1941).
[2] I. LASNITZKI, Expl Cell Res. *28*, 40 (1962).
[3] J. T. DINGLE, H. B. FELL and J. A. LUCY, Biochem. J. *98*, 173 (1966).
[4] T. C. APPLETON, Jl R. microsc. Soc. *83*, 277 (1964).
[5] N. F. CUNNINGHAM and R. A. MORTON, Biochem. J. *72*, 92 (1959).

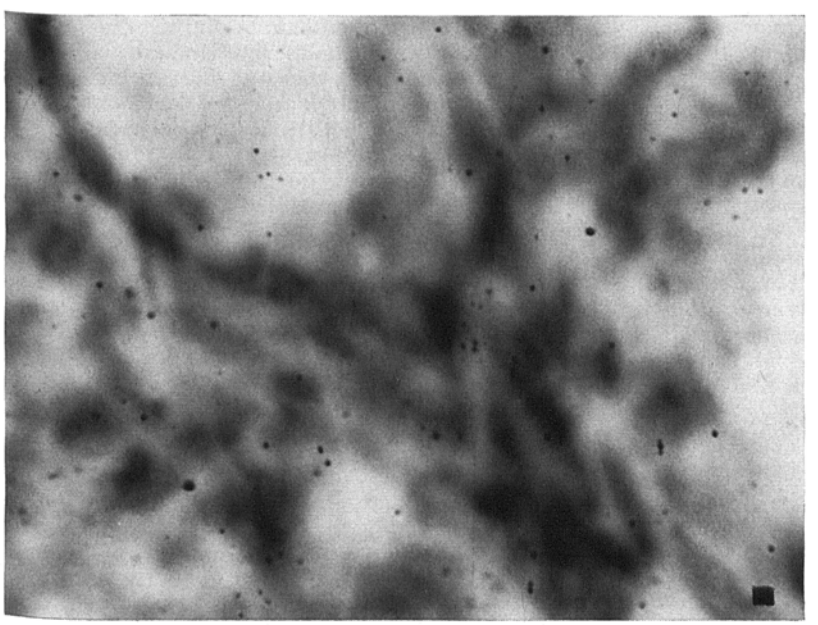

Fig. 1. Autoradiograph of ventral prostate explant. 0.3 μg/ml of C^{14}-retinyl acetate added to culture medium. Uptake after 0.5 h. Grains are seen over glandular alveolar epithelium and stromal cells. Stained with hematoxylin and eosin. × 980.

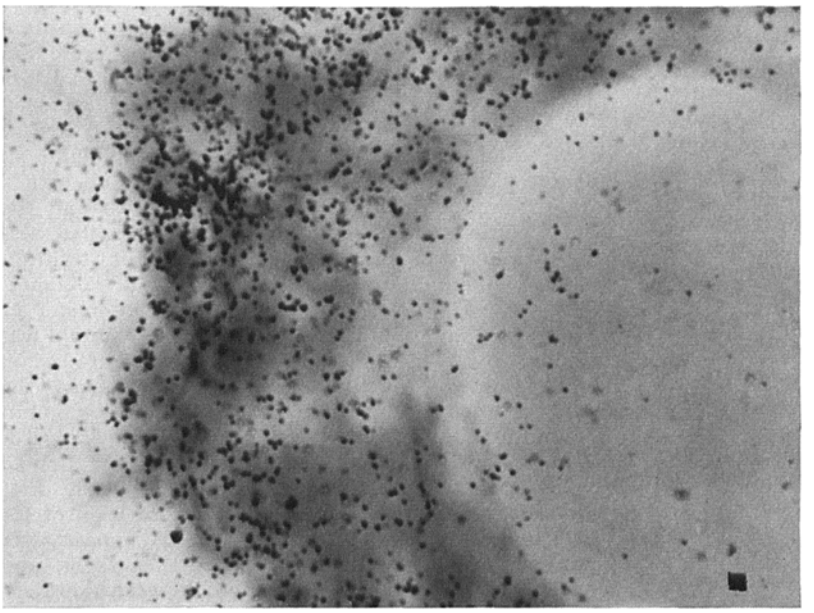

Fig. 2. Autoradiograph of ventral prostate explant. 0.3 μg/ml of C^{14}-retinyl acetate added to culture medium. Uptake after 48 h. Grains seen over the epithelial and adjacent stromal areas are considerably more numerous than before. Stained with hematoxylin and eosin. × 980.

this is true for both retinol and retinyl acetate. The amount of the vitamin found in the prostate varies within narrow limits from $1.8 \cdot 10^{-2}$ to $3.9 \cdot 10^{-2}$ μg of vitamin A/mg wet weight of tissue. No vitamin A could be detected by chemical analyses in fresh rat prostate gland.

Chemical analyses showed that initially there was $0.31 \cdot 10^{-2}$ μg/μl (4.68 μg/1.5 ml) in the medium of each culture dish. After 48 h of incubation the concentration was reduced to $0.14 \cdot 10^{-2}$ μg/μl (2.10 μg/1.5 ml). Allowing for a 30% loss of the vitamin during this period, even in the presence of the antioxidants, it is calculated that there is about a 10 to 20-fold increase in the concentration of vitamin A in the prostate explant as compared to the culture medium. This calculation is based on equating mg of tissue with μl of medium and the presence of about one mg of tissue in each culture dish.

Discussion. To the best of my knowledge, the present paper is the first to show autoradiographic illustrations of vitamin A and/or a metabolic derivative in epithelial tissue, although SIDMAN and DOWLING[6] presented a brief report on the localization of vitamin A aldehyde in the retina and STÜTTGEN and KRAUSE[7] detected tritium-labelled vitamin A by isotopic counting methods in slices of epidermis after local application. LASNITZKI and SHARMAN[8] found that after 4 h exposure to extremely high concentrations of vitamin A (120 μg/ml), the initial uptake by embryonic chick skin in vitro was approximately $5 \cdot 10^{-2}$ μg of vitamin A/mg of tissue. After 48 h of incubation in medium without vitamin A, the initially saturated chick skin still retained about $3 \cdot 10^{-2}$ μg of the vitamin/mg of tissue. These values are of the same order of magnitude as found in the present study of rat prostate explants.

The results of the present autoradiographic study indicate a progressive increase in the amount of labelled material present in the explants during the course of the experiment at both low and high dose levels. However, the analytical results suggest that the explants become quickly saturated with vitamin A during the first 30 min of exposure at the high dose level and are maintained at this concentration at a constant level for at least 48 h. This saturation is not surprising since 3.0 μg/ml represents approximately 10 times the concentration of vitamin A found in rat serum.

The differences between the autoradiographic results and those of the chemical analyses, at least at the higher dosage of vitamin A, may be interpreted in at least 2 ways, as follows: (a) the chemical methods used insufficiently extract vitamin A from the tissue: a portion of the vitamin may be so tightly bound that it is not extractable by the techniques employed or (b) the progressive increase in autoradiographic label represents conversion of labelled vitamin A to other substances which are not recognized spectrophotometrically as vitamin A by the chemical methods of analysis used in these studies. Whether these substances represent water-

Vitamin A uptake by organ cultures of rat prostate

Type of vitamin A	Time after addition of 3.0 μg/ml of vitamin A to organ cultures[a]	μg vitamin A/mg wet weight of prostate
Retinol	0.5 h	$2.4 \cdot 10^{-2}$
Retinol	1 h	$3.9 \cdot 10^{-2}$
Retinol	4 h	$2.6 \cdot 10^{-2}$
Retinol	24 h	$2.5 \cdot 10^{-2}$
Retinol	48 h	$3.0 \cdot 10^{-2}$
Retinyl acetate	0.5 h	$2.2 \cdot 10^{-2}$
Retinyl acetate	1 h	$1.8 \cdot 10^{-2}$
Retinyl acetate	4 h	$3.4 \cdot 10^{-2}$
Retinyl acetate	24 h	$2.1 \cdot 10^{-2}$
Retinyl acetate	48 h	$2.8 \cdot 10^{-2}$

[a] Since 1.5 ml of culture medium was used in each culture dish and the culture medium 199 already contains 0.1 μg of vitamin A/ml, the total amount of vitamin per culture dish should be 4.65 μg.

[6] R. L. SIDMAN and J. E. DOWLING, Anat. Rec. *145*, 286 (1963).
[7] G. STÜTTGEN and H. KRAUSE, Hautarzt *10*, 504 (1959).
[8] I. LASNITZKI and I. M. SHARMAN, Rep. Strangeways Res. Lab. 11 (1959); British Empire Cancer Report (1959).

soluble derivatives of the vitamin[9-11] or lipid/ether soluble 'active' metabolites[12-14] cannot be determined by the present experiments[15].

Résumé. Des études autoradiographiques et chimiques montrent que chez le rat, des explants du lobe ventral de la prostate incorporent de l'acétate de rétynyl pendant 48 h. Les résultats différents obtenus par les 2 méthodes utilisées indiqueraient qu'il y a non seulement incorporation de la vitamine A mais aussi transformation de cette dernière en d'autres substances.

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[9] C. F. Garbers, J. Gillman and M. Peisach, Biochem. J. *75*, 124 (1960).
[10] P. E. Dunagin Jr., E. H. Meadows Jr. and J. A. Olson, Science *148*, 86 (1965).
[11] R. D. Zachman, P. E. Dunagin Jr. and J. A. Olsen, J. Lipid Res. *7*, 3 (1966).
[12] K. Yagashita, P. R. Sundaresan and G. Wolf, Nature *203*, 410 (1964).
[13] P. R. Sundaresan, Biochim. biophys. Acta *113*, 95 (1966).
[14] M. Zile and H. F. De Luca, Biochem. J. *97*, 180 (1965).
[15] I would like to express my thanks to Dame Honor B. Fell and her associates of the Strangeways Research Laboratory, Cambridge, England where these studies were done while the author was a Visiting Scientist during 1964–1965 and to Dr. S. R. Pelc and Mr. T. C. Appleton of the Biophysics Research Unit, King's College, University of London for their advice, encouragement and generous use of their facilities. This work was supported in part by Grant No. AM 06512 from the United States Public Health Service, National Institutes of Health.

The Lipids of Human Peripheral Lymph

Investigations concerning the total fat content and the specific acid pattern of individual lipid fractions in peripheral human lymph are important for 2 reasons: (1) It is a well documented fact that ingested fat is transported from the intestinal wall mainly by means of the lymph and that therefore this biological fluid plays an important role in fat transportation. (2) Peripheral lymph is held to be a filtrate of blood plasma. The theory of simple filtration, however, which is based on the presence of pores of definite size in the capillary wall provides no explanation for the transportation of chylomicrons from peripheral blood to the lymph[1].

In the present paper some first data concerning the lipids of human peripheral lymph are presented. The results obtained can be explained partly on the basis of selective filtration and partly on that of a direct release of tissue fat into the lymph.

Materials and methods. In order to mark the lymph vessels methylene blue is injected in the interdigital fold between the first and second toe. An incision is then made in the middle third of the anterior surface of the lower leg and a lymph vessel is prepared. The vessel is cannulated so that the lymph flowing from the periphery can be collected and the cannula is held in position by ligation. Approximately 0.5–1.0 ml lymph are obtained within 1 h if the vessel is stroked or massaged gently from the periphery towards the centre from time to time. One drop of natrium citrate is added to the lymph which is then kept at -16 °C. A pool of such peripheral lymph samples obtained from 14 patients was employed in the present investigation.

The methods employed for the isolation and determination of the various lipids have been reported elsewhere[2]. Basically total fat was separated by means of silica gel column chromatography in the cholesterol, cholesterol ester, triglyceride, free fatty acid and phospholipid fractions, each of which being then quantitated gravimetrically. Subsequently, after esterification with methanol, the fatty acid pattern of each fraction was determined by means of gas liquid chromatography.

Results. In order to facilitate comparison the results of previous investigations[3] concerning the lipids in plasma and in thoracic duct lymph of fasting subjects are shown together with the data obtained in the present study from peripheral lymph in Tables I and II. As may be seen from Table I the total fat content of peripheral lymph is substantially lower than that of plasma. Similar differences are to be seen in the case of cholesterol esters and free cholesterol, whereas the differences in phospholipids and free fatty acids are not marked. The opposite situation is found where the triglycerides are concerned; here the highest values are found in the lymph of the thoracic duct and the peripheral lymph, whereas plasma values are low.

The fatty acid patterns of the various fractions in plasma, thoracic duct lymph and peripheral lymph are shown in Table II. It is evident that in the case of the cholesterol esters the highest levels of polyenoic acids are found in plasma, whereas in peripheral lymph the saturated acids predominate. The differences are particularly marked for palmitic and linoleic acid. In the case of the triglycerides the situation is reversed: the highest values for saturated acids are found in the plasma, whereas the peripheral lymph triglycerides contain relatively large amounts of highly unsaturated acids. The value for the $C_{18:2}$ content of the peripheral lymph triglycerides has been put in brackets because the corresponding peak in the gas liquid chromatogram appeared somewhat atypical with a relatively wide basis and with a 'shoulder'. In the fatty acid patterns of free fatty acids and of phospholipids, no significant differences between plasma and lymph could be demonstrated.

Discussion. The results presented in this paper are derived from 2 different groups of individuals. Nevertheless a comparison of the data may be considered permissible as (a) only the mean values are taken into consideration, (b) in both cases fasting (12 h) blood and lymph levels were estimated, and (c) both groups consisted of individuals with the same nutritional background.

The lower levels of total fat in lymph as compared to plasma could be taken as an argument in support of the theory of 'simple filtration' for the passage of fats from plasma to lymph. However the data obtained in the individual lipid fractions demonstrate that this simple

[1] J. M. Yoffey and F. C. Courtice, *Lymphatics, Lymph and Lymphoid Tissue*, 2nd edn (Edward Arnold, Publishers Ltd., London, 1956) p. 96.
[2] G. Grimmer, A. Glaser, H. Oertel, K. D. Voigt and M. Apostolakis, Hoppe-Seyler's Z. physiol. Chem. *333*, 232 (1963).
[3] M. Apostolakis, K. D. Voigt and G. Grimmer, Klin. Wschr. *43*, 1094 (1965).