TABLE I

THE EFFECT OF VITAMIN K_1 ON LIGHT-EXPOSED PARTICLES AND SUPERNATANT (3600 A)

System	Additions	Oxygen μatoms	Δ Pi μmoles	P:O
P + S	none	2.32	1.8	0.77
P + S	vit. K_1	3.46	2.7	0.81
TP + TS	none	0.62	0.3	
TP + TS	vit. K_1	2.92	3.0	1.02
TP + TS	crude heated supernatant	2.20	1.6	0.71
TP + TS	treated vit. K_1	0.78	0.2	

The system contained 0.3 ml (7.0 mg protein) of the particulate fraction (TP) treated for 1.5 h with a Gates Raymaster lamp (3600 A), 0.4 ml (17 mg protein) of supernatant (TS) treated in a similar manner, 4.0 μmoles enzymically reduced DPN, 3.9 μmoles vitamin K_1 (oil) suspended in inactive lipid, 15 μmoles $MgCl_2$, 25 μmoles KF and 7.8 μmoles inorganic phosphate. The acceptor system consisted of 2.5 μmoles ADP, 20 μmoles glucose, and 1 mg yeast hexokinase. P and S are untreated particles and supernatant, respectively. The oxygen uptake was measured at 30° for 10 min after the addition of DPNH. The reaction was stopped with 10% TCA and the mixture analyzed for phosphate disappearance.

The FMN type system appears to represent the non-phosphorylative pathway in extracts from this organism[7,9].

Vitamin K_1 is involved as a coenzyme in both electron transport and coupled oxidative phosphorylation. Preliminary evidence indicates that ~ P is incorporated in vitamin K_1. This monophosphate ester has also been postulated by WESSELS[10] to be the activated intermediate.

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Purification of a serum protein required by a mammalian cell in tissue culture

The studies of EAGLE and his co-workers on the nutrition of tissue culture cells have defined their amino acid and vitamin requirements[1]. The nature of the serum components necessary for their growth, however, has not been elucidated. A protein which is required for the growth of a tissue culture cell, human appendix – A 1*, has now been purified about 15-fold from calf serum by conventional protein fractionation procedures.

The assay procedure used to follow the purification of the protein depends upon two marked effects produced by it on washed tissue culture cells inoculated into a synthetic medium[4]. Under the conditions of the assay, in the absence of the protein factor, few cells attach to a glass surface

* Appendix – A 1 is a single clone isolate obtained in Dr. PUCK's laboratory[2] from a strain isolated by Dr. CHANG[3].

and the attached cells remain round in shape. The activities of serum and the protein fractions derived from it were estimated by measuring the smallest concentration which caused essentially all the cells to adhere to a glass surface in 14 to 16 h and induced the attached cells to assume a flattened, epithelial appearance within 24 h. Thus, in assay mixtures (1.0 ml) containing 87,000 cells, with no protein addition and with 0.75, 1.5, 2.3, and 3.0 μg of the purified fraction, 3,000, 5,000, 25,000, 79,000, and 74,000 cells adhered to the glass surface, respectively. With no protein and with 0.76 μg of protein no flattening of the cells occurred while with 2.3 and 3.0 μg of protein the cells flattened and the population microscopically resembled a healthy growing culture. With 1.5 μg of protein, flattening of some cells occurred but many cells remained rounded.

Other sera tested, human, guinea pig, and rabbit, also caused adherence to a glass surface and flattening of the tissue culture cells.

The activity is not dialyzable, it is insoluble in water but soluble in salt solutions, and it is heat-labile. After heating the purified preparation (pH 6.8) for 10 min at 50, 60, and 70° C, the recoveries of the activity, as measured both by attachment to a glass surface and by the microscopic appearance of the cells, were 60, 15, and less than 8%, respectively. The purified preparation is comprised mainly or entirely of glycoprotein; the content of hexose, hexosamine, and sialic acid*, per mg of protein is 0.049, 0.036 and 0.043 mg, respectively.

For cell multiplication, in addition to the protein, a heat stable, dialyzable factor(s) is also required. This factor was provided as a dialysate of an autoclaved solution of peptone (Difco) (Table I).

TABLE I

GROWTH WITH THE PURIFIED SERUM PROTEIN

The cells used in this experiment had been grown in a medium containing the protein fraction and peptone in place of serum. 0.5 ml aliquots of a cell suspension (35,000 cells) which had been twice washed with basal medium were added to 2.0 ml basal medium containing the indicated additions. Growth was measured by estimating cell numbers with a Levy counting chamber after incubation for 110 h at 36° C.

Additions per ml	*Number of cells*
None	33,000
Peptone dialysate (0.04 ml)	39,000
Purified protein (60 μg protein)	67,000
Purified protein (60 μg protein) + peptone dialysate (0.04 ml)	168,000
Calf serum (1,300 μg protein)	64,000
Calf serum (3,250 μg protein)	103,000
Calf serum (6,500 μg protein)	156,000

In the ultracentrifuge, the purified preparation shows two components with similar sedimentation constants ($S_{20,w}$ about 7), the major component representing about two-thirds of the total protein. Electrophoretic analysis also reveals two components which appear to move as *α-globulins*.

The exact nature of the protein and the role it plays in the metabolism of the tissue culture cell must await further purification. It is interesting to speculate, however, that the protein serves as a carrier for a sugar derivative which cannot be synthesized by the mammalian cell.

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* Hexose and hexosamine were estimated according to WINZLER[5], sialic acid directly with Ehrlich's reagent[6] and protein by the procedure of LOWRY *et al.*[7].