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15. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

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9 October 1956

Contamination of Human Cell Cultures by Pleuropneumonialike Organisms

In the course of a study to determine the effect of pleuropneumonialike organisms of human and animal origin on various types of cell cultures available in the laboratories of the department of microbiology, School of Hygiene and Public Health, Johns Hopkins University, a human strain of pleuropneumonialike organisms was inoculated into tubes of HeLa cells. After 48 hours of incubation the fluid medium was removed from the inoculated and the uninoculated control HeLa cell cultures, and 0.1 ml of each was planted onto plates of pancreatic digest agar, using the method previously described for cultivation of pleuropneumonialike organisms (*1*). Growth of typical colonies of pleuropneumonialike organisms was noted on all plates, including the plates inoculated with the fluid from the control HeLa cell culture. This finding was checked and found to be reproducible.

Careful analysis of the experimental design has since shown that the isolated strain of pleuropneumonialike organisms could not have been introduced at the time at which the experiments were carried out. Following this observation, two other sublines of HeLa cell cultures maintained in the same laboratory by other workers, as well as a subline of conjunctival cells maintained in this laboratory, and a culture of HeLa cells recently purchased from a commercial laboratory were examined. The results are summarized in Table 1. It was estimated that there were 1000 to 5000 pleuropneumonialike organisms per milliliter of culture fluid in the positive HeLa cell cultures and 3000 to 10,000 organisms per milliliter in the human conjunctival cell culture (Fig. 1). The organisms from both sources grew well in subculture in liquid as well as on solid media. Growth in liquid media produced no turbidity.

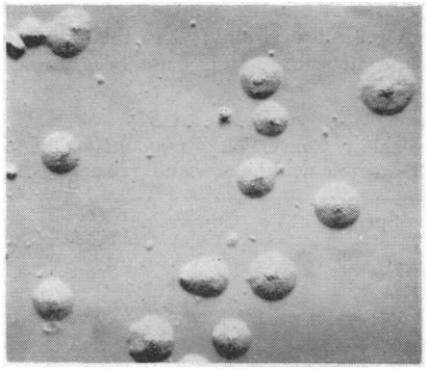

Fig. 1. Photomicrograph of colonies of pleuropneumonialike organisms from human conjunctival cell culture. (× 75)

It appears that sometime between October 1955 and April 1956 the main sublines of cell cultures became contaminated with pleuropneumonialike organisms, probably through some common constituent of culture media. It is of interest that for some time within this period, focal necrotic areas and considerable granulation of cells were frequently manifest.

Contamination of a tissue culture with an unknown agent later identified as pleuropneumonialike organisms has been encountered previously by other workers (*2*). Pleuropneumonialike organisms may easily escape detection or recognition unless they are specifically looked for. Their colonies are microscopic. In liquid media many strains, particularly those of human origin, produce either no turbidity or turbidity which is so slight that it is not readily apparent to the naked eye. They are filtrable microorganisms (*3*) and, therefore, are not removed by most routine filtration procedures used in the preparation of tissue-culture media. They survive freezing. Penicillin in concentrations customarily added to tissue-culture media has no effect on them. The effect of streptomycin of similar levels is variable, depending on the sensitivity of individual strains. The majority of strains of pleuropneumonialike organisms isolated from various sources are sensitive to antibiotics of the tetracycline series (*1*). The strains isolated from the HeLa cell cultures and from the subline of conjunctival cells were found to be sensitive to 4 μg oxytetracycline per milliliter and 256 μg streptomycin per milliliter.

Information regarding metabolic activities and nutritional requirements of members of the pleuropneumonia group is still incomplete (*4*). Recently a few data have become available regarding the effect of these organisms on living tissues. Potential cell parasitism and cell damage are suggested by the demonstration of intracytoplasmic inclusions due to pleuropneumonialike organisms (*5*) and by the abnormal appearance of the HeLa cell cultures examined by us. The undetected presence of pleuropneumonialike organisms may, therefore, invalidate or confuse observations made on presumably uncontaminated cultures.

Table 1. Data on cell cultures examined for presence of pleuropneumonialike organisms (*6*).

Cell culture	Growth of pleuropneumonialike organisms	Source	Maintenance
HeLa 1	+	JH*	Routine† by A
HeLa 2	+	JH*	Routine by B
HeLa 3	+	JH*	Routine by C
HeLa 4	0	JH*	Frozen October 1955
HeLa 5	0	CL	Obtained May 1956
Conjunctival	+	JH	Routine‡

* Strain originally obtained from CL (Commercial Laboratory) in summer of 1955, maintained at the department of microbiology, School of Hygiene and Public Health, Johns Hopkins University.
† HeLa cells: 40-percent human serum, 60-percent Hank's saline, penicillin 100 units/ml, streptomycin 100 μg/ml.
‡ Conjunctival cells: 10-percent human serum, 90-percent Eagle's medium, penicillin 100 units/ml, streptomycin 100 μg/ml.

The findings presented here indicate that tissue cultures or the components of tissue-culture media may be contaminated with pleuropneumonialike organisms and that this contamination is not readily apparent. Routine or at least frequent checks of the cultures and media for these organisms should be considered. In addition, the incorporation of a suitable level of one of the antibiotics of the tetracycline series may aid in the prevention of contamination by pleuropneumonialike organisms.

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6. Since this paper was submitted for publication, nine additional sublines of cell cultures were checked for contamination with pleuropneumonialike organisms. One of the sublines yielded growth of pleuropneumonialike organisms.

8 October 1956

Comparative Studies of Lipoproteins by Starch and Paper Electrophoresis

Previous investigations have been concerned with the cholesterol and phospholipid content of serum lipoproteins separated either by paper electrophoresis (*1*) or by starch electrophoresis (*2–4*). This study is concerned with comparative analyses of cholesterol and phospholipid in the various serum lipoproteins separated simultaneously by starch and paper electrophoresis (*5*). Normal serum and serums from patients with idiopathic hypercholesteremia and with idiopathic hyperlipemia were analyzed.

Starch electrophoresis was performed in two parallel blocks 8 by 36 by 1.5 cm using 2.0 ml of serum for each block and applying 450 volts and a current of 18 to 35 ma for 16 to 18 hours in a cold room at a steady temperature of 5°C. Barbiturate buffer of *p*H 8.6 and of ionic strength 0.05 was used. Extraction of the

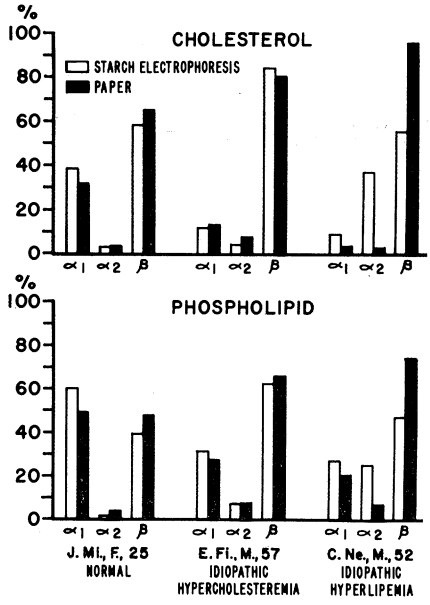

Fig. 1. Cholesterol and phospholipid contents in alpha-1, alpha-2, and beta lipoprotein fractions separated by starch and paper electrophoresis. No significant differences were observed between the two methods of electrophoresis in a normal person or, in persons with idiopathic hypercholesteremia. In idiopathic hyperlipemia, marked increase of cholesterol and phospholipid was seen in the alpha-2 lipoprotein by starch electrophoresis, whereas, by paper electrophoresis, these lipids were mainly increased in the beta lipoprotein.

starch segments (1 cm wide) was performed with a mixture of chloroform and methanol (2/1). The extracts were analyzed for total and esterified cholesterol (*6*) and phospholipid (*7*). Analogous segments of the second block were used for protein determinations by the biuret method (*8*).

Paper electrophoresis was performed in two parallel strips of Whatman 3-MM filter paper (15 by 30 cm) using 0.4 ml of serum and applying 250 volts and a current of 15 to 20 ma for 7 hours. A 2-cm-wide part of one strip was stained with Amidoblack 10B dye for localization of the protein fractions. The buffer and the lipid solvent were identical with those used in starch electrophoresis. Each paper segment (1 cm wide) was analyzed for total and esterified cholesterol and phospholipid (*6*, *7*).

The control serum showed normal lipid partition: cholesterol, 228; phospholipid, 304; triglycerides, 28; and total lipids, 560 mg percent. The lipoprotein fractions alpha-1, alpha-2, and beta that were separated either by starch or by paper electrophoresis showed no significant differences in the distribution of cholesterol and phospholipid (Fig. 1).

In idiopathic hypercholesteremia, the serum was characterized by decided elevation of both cholesterol and phospholipid with a slight elevation of triglycerides and total lipids: cholesterol, 334; phospholipid, 328; triglycerides, 118; and total lipids, 880 mg percent. Regardless of the method of electrophoresis used, the cholesterol and phospholipid contents were markedly increased in the beta lipoprotein fraction and decreased in the alpha-1 lipoprotein fraction (Fig. 1).

In idiopathic hyperlipemia, the serum was characterized by lactescence and by marked elevation of all lipid fractions: total cholesterol, 776; phospholipid, 764; triglycerides, 1460; and total lipids 3000 mg percent. Marked differences in the distribution of cholesterol and phospholipid were observed with the two methods of electrophoresis. When starch was used as the supporting medium, elevation of cholesterol and phospholipid in the alpha-2 lipoprotein fraction was the prominent feature, whereas when paper was used the elevation of these lipids was seen in the beta lipoprotein (Fig. 1). By both methods, decrease of these lipids was observed in alpha-1 lipoprotein.

Considerable adsorption of serum triglycerides (chylomicrons) to the paper was noted at the point of application (*2*). A comparison of the distribution curves of cholesterol and phospholipid by the two methods of electrophoresis showed that large amounts of cholesterol and phospholipid were present in both alpha-2 and beta lipoprotein when starch electrophoresis was used, while the major pattern of these lipids was found in the beta lipoprotein by paper electrophoresis.

The adsorption of serum lipids at the point of application in paper electrophoresis interfered with the migration of some of the cholesterol and phospholipid molecules. When starch was used as supporting medium, no accumulation and adsorption of triglycerides was observed at the point of origin. The triglycerides migrated freely (*4*) and the cholesterol and phospholipid molecules migrated with them. This observation is probably related to the easier extractability of cholesterol "enmeshed in lipids" (*9*).

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31 July 1956

Missing Step in Guinea Pigs Required for the Biosynthesis of L-Ascorbic Acid

Man, other primates, and guinea pigs are the only mammals known to be unable to synthesize L-ascorbic acid; thus they require vitamin C in their diet to prevent scurvy. It has not been known

Table 1. Conversion of L-gulonolactone to L-ascorbic acid by rats and guinea pigs.

Species	Conversion* (%)
Rat	9.1
Rat	7.2
Guinea pig	< 0.2
Guinea pig	< 0.2

* Estimated from the amount of C^{14}-labeled L-ascorbic acid present in the animal 24 hours after intraperitoneal administration of 12-mg doses of L-gulonolactone-1-C^{14} (*3*).