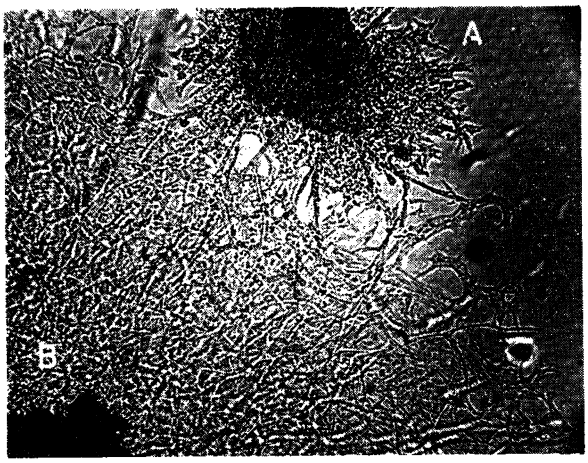

FIG. 1. Four-day combined culture of (A) spleen of mouse immunized with chicken serum, and (B) 1-day-old chick heart. Note that outgrowth zones have joined and that there is no evidence of antagonism. Magnified 90 ×.

During the first few days of incubation, extensive cellular migration occurred from all explants. In cultures of tissues from different species, cells at the margins of the outgrowth zones came into intimate contact with each other (Figs. 1 and 2) as in cultures of tissues from the same species. In both types of culture, the cells in the contact zone, as elsewhere, appeared normal. The cells were observed to come together in the contact zone and, as migration continued, the margins of the outgrowth zones were joined without any sharp demarcation. There was no evidence of: 1) abnormal cellular accumulation or reaction along the line of junction between the two explants, 2) altered rate or direction of growth in the contact area, or 3) specific attraction or antagonism between cells of the two species. Once the growth zones joined, continued cultivation with or without embryo juice, up to a maximum of 21 days, produced no further change, apart from progressive growth of the cultures.

The growth patterns described were identical in the case of combinations involving explants from previously immunized animals. Tissues of immunized mice, as compared with those of nonimmunized mice, showed no difference in reaction to tissue fragments from the species that furnished the antigens.

The results show that tissues of two different species, as widely diverse as mouse and chicken, may be grown simultaneously in flask culture without apparent antagonism. This is true even when the donor mouse has been previously immunized against antigens of the species furnishing the other tissue of the paired combination, and when the mouse tissue is one which *in vivo* presumably is intimately involved in antibody formation, i.e., spleen and lymph node. At present, the chief significance of these findings is seen in the possibility that such combined cultures may provide a method for investigations requiring maintenance of healthy tissues of two species in close physiological relations.

Further investigation is necessary before these results may be interpreted in relation to problems of antibody production *in vitro* and of incompatibility in transplantation *in vivo*. Interpretation at present is hindered for the following reasons: First, no attempt was made to provide optimum conditions for antibody production, or its detection in the culture fluids. Second, the media used contained components heterologous for both species, thereby complicating immunological interpretations. Finally, in the present state of knowledge of cellular physiology in culture, it is not safe to transfer directly to the organism conclusions based upon observations of cellular behavior *in vitro*.

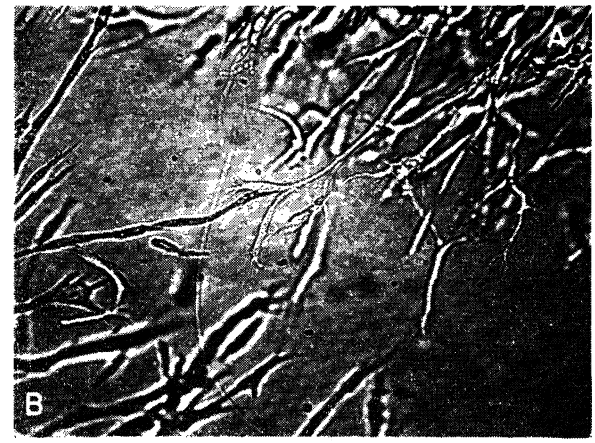

FIG. 2. Four-day combined culture of (A) lymph node of mouse immunized with guinea pig serum, and (B) 3-day-old guinea pig kidney. Intimate contact of normal cells from the two species can be observed. Magnified 280 ×.

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Transphosphorylation by Alkaline Phosphatase in the Absence of Nucleotides[1]

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Axelrod (*1*), using citrus fruit phosphatase, observed the transfer of phosphate from nitrophenyl phosphate to methanol in the absence of nucleotides. The transferred phosphate did not pass through the inorganic stage. Recently, we observed with alkaline intestinal phosphatase a strong acceleration of the rate of synthesis of glycerophosphate from inorganic phosphate and glycerol, in the

[1] This work was aided by grants from the American Cancer Society, recommended by the Committee on Growth; the Division of Research Grants and Fellowships of the National Institutes of Health; and the Rockefeller Foundation.

presence of biological compounds with phosphate bonds of higher energy than glycerophosphate (*2*).

It has since been established that this acceleration is achieved in the absence of nucleotides by a direct phosphate transfer, that is, without passing through the intermediary stage of inorganic phosphate. When a mixture of P^{32}-labeled phosphocreatine, synthesized enzymatically (*3*), unlabeled inorganic phosphate, and glycerol was incubated with intestinal phosphatase at 38° C for 15 and 30 min, the glycerophosphate in excess of that synthesized in the absence of phosphocreatine (representing two-thirds to three-fourths of the total glycerophosphate) had about the same specific activity as the phosphocreatine, while the specific activity of the inorganic phosphate was quite low. If the phosphate transfer had passed through the intermediary stage of inorganic phosphate, then the specific activity of the glycerophosphate synthesized could not be higher than that of the inorganic phosphate. Similar results were obtained with radioactive phosphocreatine, fructose, and inorganic phosphate: at the end of 15 min of incubation more than half of the total fructose-phosphate formed derived its phosphorus directly from the phosphocreatine. Other observations indicate that phosphopyruvate glucose-1-phosphate (and similar compounds with relatively high phosphate bond energy) can also participate as phosphate donors in such a direct phosphate transfer.

TABLE 1

SPECIFIC ACTIVITIES OF THE PHOSPHATE SPECIES IN THE PHOSPHORYLATION OF GLYCEROL AND FRUCTOSE AT 38° C WITH PHOSPHOCREATINE LABELED WITH P^{32}

	Initial molar concentration	% Acceleration of synthesis	Specific activity cpm/γ P	
A			0 min	30 min.
Phosphocreatine	0.0252		1090	895
Inorganic P	0.448		0	9.3
Glycerol	1.63		–	–
Glycerophosphate	0.0	290	0	606*
B			0 min	15 min
Phosphocreatine	0.030		1828	1750
Inorganic P	0.444		0	13.3
Fructose	2.34		–	–
Fructose-phosphate	0.0	375	0	730*

* Corrected for the nonlabile P impurities present in the phosphocreatine preparation.

In Table 1 the specific activities (cpm/γ P) of the different phosphate species from two such experiments are given. The general composition of the enzymatic incubation mixture was the same as previously reported (*2*).

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Hatching Eggs of Floodwater Mosquitoes in Media that Promote Plant Growth[1]

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In a natural environment, eggs of floodwater mosquitoes are deposited on soil, in debris, or among plants in places subject to transient submergence. In such sites they remain in a viable state for a year or longer, and usually hatch when they are flooded with water at a proper temperature. However, submergence in this manner is not a dependable provocation for hatching eggs of these mosquitoes in the laboratory, according to numerous observers. Water in natural habitats must contain stimulants to hatching that are not found in tap water. Several observers have noted that durable eggs of the genus *Aedes* may be stimulated to hatch by infusions made from plants that grow in the natural oviposition sites and by cultures of bacteria and yeasts. Abdel-Malek (*1*) found that eggs of *Aedes trivittatus* Coq. hatched erratically in dilute solutions of chemicals that regulate plant growth such as 1-naphthaleneacetic, 3-indoleacetic, and 3-indolebutyric acids. No one seems to have devised a way to get eggs to hatch in consistently high percentages in a few hours, as occurs in nature.

Larvae of floodwater mosquitoes hatch after two phases of growth have been completed in the egg. The first is an increase in number of cells, which continues until the fully formed embryo occupies all of the interior of the egg. The second is an increase in size of the embryo, until the shell of the egg is ruptured. Between the two periods, eggs may be dormant for months. Temperature regulates the rate of increase of cells, and the nature of the solution surrounding the eggs has much to do with initiating escape of the larvae. The final act of hatching involves an increase in size of the embryo in a manner similar to the elongation phase of growth of plant tissue. Media that stimulate one might affect the other similarly.

Eggs of the floodwater species, *Psorophora discolor* (Coq.) may hatch at any time after the embryos are fully formed. Maturation of the embryo requires about 4 days at a temperature between 22 and 26° C. If kept on a moist surface at a temperature within this range, larvae may hatch whenever the eggs are submerged in a suitable medium. Eggs kept on a moist substratum at a temperature of 15–20° C in the laboratory have survived and yielded vigorous larvae after at least 9 months. However, prolonged exposure of eggs to temperatures as low as 15–20° C will prevent any medium from causing hatching until the eggs are conditioned for several days at a temperature favorable for hatching.

Substances that promote growth of plant tissues vary in effectiveness. Thimann (*3*) states that short sections of etiolated oat coleoptiles will elongate slightly in water, more in water containing purified growth-promoting sub-

[1] Contribution No. 290 of the Department of Entomology, University of Illinois, Urbana.