concerned with the virulence of rickettsiae, and perhaps of other infectious agents, and may also give valuable clues as to how infectious agents persist in nature. It might be mentioned that the avirulent phase of *R. rickettsii* has properties similar to those of masked viruses (*8*), in that a non-infective state exists although the parasites can be made infective by the appropriate procedures. If this phenomenon turns out to be more common than is now thought, it must be taken into account in any study of the natural history of an infectious agent. In such studies the presence or absence of the agent is usually determined by infectivity measurements. However, in view of the above results the failure to find an infective agent by infectivity determinations would not necessarily mean that the agent is not present.

Enzyme Studies. In the final analysis, the difference in the virulence of various strains is probably due to a difference in their enzyme systems. Experiments were therefore carried out on strains R, S, T, and U described in Table 1, to see if any enzyme difference could be found. Because Bovarnick and Miller (*9*) and Wisseman *et al.* (*10*) have found part of the Krebs cycle to be functioning in *R. mooseri,* this system was studied first.

No differences have been found so far in any of the enzyme systems of the various strains. All strains of purified rickettsiae (*cf.* Table 1 for procedure) oxidize glutamate, pyruvate, α-ketoglutarate, succinate, fumarate, malate, and oxalacetate. The oxidation of pyruvate and glutamate is greatly stimulated by the addition of inorganic phosphate. In three experiments of seven using carefully washed rickettsiae, a definite phosphorus uptake was observed when glutamate was oxidized. No phosphorus was taken up anaerobically. It would seem that in *R. rickettsii* the oxidation of substrates is coupled to phosphorylation, as it is in other types of cells. When glutamate is oxidized, succinate accumulates in the presence of malonate. Although no oxidation occurred with added citrate, isocitrate, or *cis*-aconitate, preliminary evidence indicates that the addition of pyruvate and oxalacetate leads to the accumulation of citrate in the presence of fluoroacetate, coenzyme A, adenosine triphosphate coenzyme 1, α-lipoic acid, and glutathione. If this result is substantiated by future experiments, the indication is that a tricarboxylic acid is concerned in rickettsial metabolism. None of the reactions listed above was found in normal yolk sacs treated by the procedure used to isolate the rickettsial preparations.

Although rickettsial preparations purified by the celite and albumin procedure have been found to contain phosphatase and other enzymatic activities, treatment of such preparations with antisera prepared to normal yolk sac material gets rid of these enzymes activities, leaving only the Krebs cycle reactions mentioned above. Rickettsial preparations purified to such a degree that no host material can be detected serologically contain both pentosenucleic acid and deoxyribonucleic acid.

Details of all this work will be published in future papers.

References

1. PARKER, R. R. *Bull. Montana State Board of Health, No. 26,* 33 (1923) ; PARKER, R. R. Unpublished.
2. SPENCER, R. R., and PARKER, R. R. *U. S. Public Health Repts.,* **39**, 3027 (1924).
3. WISSEMAN, C. L., JACKSON, E. B., HAHN, F. E., LEY, A. C., and SMADEL, J. E. *J. Immunol.,* **67**, 123 (1951).
4. BACKUS, K. C., and WILLIAMS, R. C. *J. Applied Phys.,* **21**, 11 (1950).
5. HENLE, W. *J. Exptl. Med.,* **90**, 1 (1949).
6. PHILIP, C. B. Personal communication.
7. PRICE, W. H. Unpublished.
8. SHOPE, R. E. *J. Exptl. Med.,* **77**, 127 (1943).
9. BOVARNICK, M. R., and MILLER, J. C. *J. Biol. Chem.,* **184**, 661 (1950).
10. WISSEMAN, C. L., HAHN, F. E., JACKSON, E. B., and SMADEL, J. E. *Federation Proc.,* **10**, 424 (1951).

Manuscript received July 16, 1952.

Inductive Epithelio-mesenchymal Interaction in Cultured Organ Rudiments of the Mouse

Clifford Grobstein[1]

National Cancer Institute,
National Institutes of Health, Bethesda, Maryland

The importance of inductive interaction between tissues of the developing embryo has been amply demonstrated but the mechanisms remain obscure (*1*). Studies of inductive processes in the mammalian embryo have been extremely limited due to technical difficulties. Recently it has been found that very early rudiments of the mouse submandibular salivary gland (*2*), kidney, and lung (*3*), continue surprisingly normal morphogenesis at the glass-clot interface in Carrel flasks, and that each of these rudiments can be separated into viable epithelial and mesenchymal components by exposure to trypsin—as has been described for chick rudiments by Moscona and Moscona (*4*). These procedures have allowed study of epithelio-mesenchymal interaction by observing the behavior *in vitro* of heterogeneous combinations, e.g., submandibular epithelium and kidney mesenchyme (nephrogenic cord), or kidney epithelium (ureteric bud) and submandibular capsular mesenchyme. The results of one such experiment are reported here to indicate that: (1) the method affords approaches to problems of inductive interaction of embryonic tissues; (2) as has been inferred on indirect evidence (*5*), inductive processes similar to those described in lower vertebrates also occur in mammals; (3) even in the relatively simple epithelio-mesenchymal systems considered here, at least two types of inductive processes appear to be operating.

All embryos were BALB/C × C3H F_1 hybrids. Under a dissecting microscope, the submandibular rudiments were isolated on the 13th day of pregnancy (observation of a vaginal plug = 0 day), kidney rudi-

[1] With the technical assistance of Derrell Freese, George Parker and Edward J. Soban.

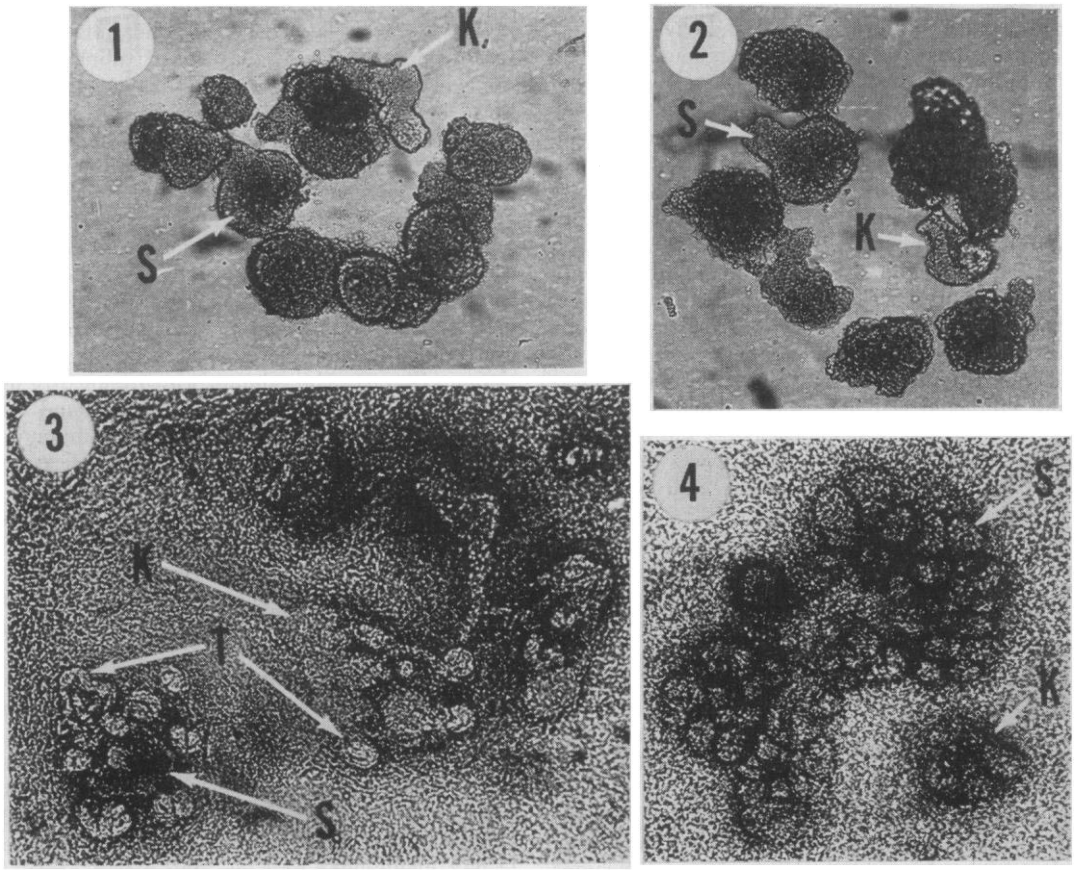

FIG. 1. One kidney (K) and one submandibular (S) epithelial rudiment combined with a number of kidney mesenchyme fragments. Just after explanation. (× 50.)

FIG. 2. As Fig. 1, except mesenchyme is from submandibular capsule. (× 50.)

FIG. 3. Culture in Fig. 1 after 3 days. Note the branching kidney epithelium, with associated early coiled tubules (T) derived from the rudiment on the right in Fig. 1; and the nondeveloping submandibular epithelium, with closely associated coiled tubules, derived from the rudiment on the left in Fig. 1. (× 50.)

FIG. 4. Culture in Fig. 2 after 4 days. (× 50.)

ments on the 11th day. At these stages both rudiments consist of a simple epithelial component expanded distally preparatory to branching, and a surrounding mesenchymal component distinguishable from the generalized loose mesenchyme. In the case of the kidney, the "mesenchymal" component is the posterior end of the nephrogenic cord; in the case of the submandibular gland, it appears to be a condensation of the ordinary loose mesenchyme.

The isolated rudiments were placed in covered tissue dishes in a 1:1 mixture of horse serum and Tyrode's solution, in an atmosphere containing 5% CO_2. The latter precaution prevents rise of pH in the fluid with resulting stickiness of the mesenchyme. Pairs of rudiments were transferred to 3% trypsin (Difco 1:250) in Tyrode's solution minus calcium and magnesium salts as recommended by Moscona (*4*). Neither preliminary exposure to Ca-Mg-free Tyrode's, nor incubation during exposure were found necessary for present purposes. After 5–10 min the rudiments were flushed in and out of an orally controlled pipet of diameter slightly less than that of the rudiment. Adhering loose mesenchyme dissociated readily and was sheared off of the rudiment as individual cells or small clusters. With continued flushing and gentle manipulation with a cataract knife, the epithelium and condensed mesenchyme separated—the former intact, the latter either intact, or in several large fragments. These components, when transferred to the horse-serum-Tyrode's mixture, showed little tendency to dissociate further and could be put in culture in small clusters in any combination desired. The condensed mesenchyme from each rudiment was separated before culture into 4 fragments for greater convenience in arranging clusters around the epithelium.

Culture was in Carrel D-3.5 flasks at the glass-clot interface. The tissue fragments were quickly oriented in a clotting mixture of rooster plasma (0.6 cc) and nutrient medium (0.8 cc) consisting of horse serum, Tyrode's solution and chick-embryo juice (9 day) in

2:2:1 ratio. After clotting, a supernatant of 1 cc of the nutrient medium was added and changed daily. Aseptic procedures were used throughout. The cultures were incubated at 37–38° C. Daily observations and frequent photographic records enabled the developmental behavior of each individual epithelial rudiment to be followed.

In the experiment under consideration 5 pairs of cultures were prepared. In one flask of each pair, one submandibular and one kidney epithelial component were placed in a close cluster of submandibular capsular mesenchyme fragments (Fig. 2). In the other flask, similar epithelial components from the opposite sides of the same embryos were combined with kidney mesenchyme fragments (Fig. 1). In each flask, therefore, one epithelial rudiment was in homogeneous recombination with its own mesenchyme, the other was in heterogeneous combination. In all cases the mesenchymal fragments rapidly spread and coalesced around both epithelial rudiments.

In all flasks involving submandibular capsular mesenchyme the submandibular epithelium underwent its characteristic morphogenesis as previously noted in cultured intact rudiments (Fig. 4) (*2, 6*). The kidney epithelium, however, rounded up and showed no characteristic kidney morphogenesis. In several cases its general configuration was somewhat suggestive of submandibular epithelium, although there was no formation of adenomeres or other indication of growth and morphogenesis. No significance can be attached to this latter point pending further data. For the moment it can only be said with certainty that kidney epithelium in submandibular capsular mesenchyme fails to show its own characteristic morphogenesis.

On the other hand, this same epithelium in kidney mesenchyme did undergo characteristic morphogenesis. It formed a branching pattern of ducts during the first several days followed, beginning on about the third day, by the appearance of coiled tubules in the kidney mesenchyme at the periphery of, and among, the ducts (Fig. 3). Similar coiled tubules appear in intact 11-day metanephric rudiments cultured under these same conditions (Fig. 5) and appear to represent rudiments of the secretory portion of the uriniferous elements, formed *in vivo* by the nephrogenic cord and secondarily linked to the collecting system arising from the ureteric bud. In the cultures involving kidney mesenchyme, the submandibular epithelial components completely failed to undergo their characteristic morphogenesis, rounding up to form small masses. In close association with these masses, however, coiled tubules developed in the kidney mesenchyme (Fig. 3). The tubules, which tended to develop earlier, and become more prominent around the submandibular rudiment than around the kidney epithelium, were not seen except in close association with one or the other epithelia.

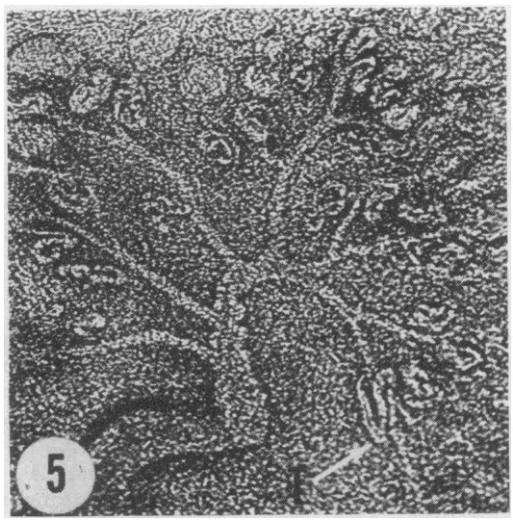

FIG. 5. Metanephric rudiment of 11-day embryo on 8th day in culture. Note branching system of collecting tubules with intercalated coiled tubules. (× 50.)

The results in all flasks, although quantitatively variable, were qualitatively the same and were duplicated when the two types of epithelium were tested separately rather than in the same flask. The implication of the results that submandibular and kidney epithelial morphogenesis depends upon specific properties in the associated mesenchyme is supported by other experiments showing that submandibular epithelium fails to undergo morphogenesis when combined with 11-day mandibular arch mesenchyme, 11-day lung mesenchyme, 11-day limb-bud mesenchyme or 8-day somite and lateral plate mesoderm. Similarly, kidney epithelium fails to undergo morphogenesis in 11-day mandibular arch or lung mesenchyme. Moreover, submandibular capsular mesenchyme cultured separately and recombined with submandibular epithelium at each of 3 successive subculture periods of 5–7 days continues to exert a recognizable morphogenic effect, although of declining degree and typicalness.

It would appear, from the reported observations, that tubule formation by nephrogenic cord is less specific in its inductive requirements than morphogenesis of the epithelia tested. Whereas the latter occurs *in vitro* only in the particular mesenchyme normally surrounding the epithelial rudiment in the embryo, the former can be induced by submandibular epithelium *in vitro* and by spinal cord both *in vivo* (*7*) and *in vitro* (*3*). That the effect is not completely nonspecific, however, is suggested by preliminary experiments indicating at least much reduced activity of killed spinal cord and inactivity of 11-day lung, pancreas, and stomach epithelia (*3*).

It is of interest to note that the suggestion of two types of inductive processes seemingly differing in their specificity is reminiscent of data on amphibian primary induction where workers have drawn a distinction between simple nonspecific "evocative" and more complex and more specific "individuation" effects (*8*). Although that situation and the present one are not completely comparable, it is suggestive that primary induction, too, involves an epithelio-mesen-

chymal system. Continued analysis of such systems, isolated and controlled in culture, may reveal fundamental similarities in the patterns of inductive interaction in the whole early embryo and its later subsystems.

References

1. HOLTFRETER, J. *Growth*, Symposium Vol. X, 117 (1951).
2. GROBSTEIN, C. *J. Morphol.*, **93**, (1), (1953).
3. ———. Unpublished data.
4. MOSCONA, A., and MOSCONA, H. *J. Anat.*, **86**, 287 (1952); ———. *Expti. Cell Research*, **3**, 535 (1952).
5. GLUECKSOHN-SCHOENHEIMER, S. *Growth*, Symposium Vol. IX, 163 (1949).
6. BORGHESE, E. *J. Anat.*, **84** 287 (1950).
7. GRUENWALD, P. *Anat. Record*, **86**, 321 (1943).
8. WADDINGTON, C. H. *Organizers and Genes.* Cambridge: Cambridge Univ. Press (1940).

Manuscript received December 30. 1952.

The Use of Some Synthetic Phosphatides in Antigens for the Serodiagnosis of Syphilis

D. B. Tonks and R. H. Allen

Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada

The cardiolipin antigens used in the serodiagnosis of syphilis contain 3 components: cardiolipin, lecithin, and cholesterol. Cardiolipin is purified from extracts of beef heart and the lecithin may be prepared from beef heart or egg yolk. The substitution of synthetic compounds in place of these naturally occurring components was suggested to us with the synthesis of saturated lecithins by Baer and associates (*1, 2*).

structure to cardiolipin, has been studied as a cardiolipin substitute. Antigens containing the various synthetic substitutes have been used in the V.D.R.L. microflocculation test and the Kolmer complement fixation test (Table 1).

Three α-lecithins of the L series, namely, distearoyl, dipalmitoyl, and dimyristoyl lecithin were studied. Distearoyl lecithin is sparingly soluble in ethyl alcohol, the basic solvent of cardiolipin antigens. When used in place of beef-heart lecithin in antigen for the V.D.R.L. slide test, the resultant mixture was undersensitive even at the highest possible concentration. Again, in the Kolmer test, only a few antigens could be prepared for trial, but one of these was found to possess a sensitivity quite close to that of Kolmer antigen.

Many mixtures prepared with the more soluble dipalmitoyl and dimyristoyl lecithins were used in the V.D.R.L. microflocculation test, and it was found that the most sensitive antigens had the following composition: cardiolipin, 0.03%; synthetic lecithin, 0.3%; cholesterol, 0.9%. Antigen suspensions prepared from these mixtures were found to increase in sensitivity for the first 4 hr, and then remained constant for 24 hr. If heated for 5 min at 56° C, however, a stable suspension of the same maximum sensitivity was obtained immediately. The most reactive preparations were not quite equal in sensitivity to our V.D.R.L. slide-test antigen. These lecithins were also used in the Kolmer test and levels of sensitivity close to that of Kolmer lipoidal antigen were obtained when mixtures of the following composition were used: cardiolipin,

TABLE 1

SUBSTITUTION OF SYNTHETIC PHOSPHATIDES FOR CARDIOLIPIN OR NATURAL LECITHIN IN THE PREPARATION OF ANTIGENS FOR THE SERODIAGNOSIS OF SYPHILIS

Cardiolipin	Lecithin	V.D.R.L. microflocculation test	Kolmer complement fixation test
Tetramyristoyl-bis-(L-α-glyceryl) phosphoric acid		Weakly reactive	Reactive
	L-α-distearoyl lecithin	Weakly reactive	Reactive
	L-α-dipalmitoyl lecithin	Reactive	Reactive
	L-α-dimyristoyl lecithin	Reactive	Reactive
	D-α-dimyristoyl lecithin	Reactive	Reactive
	DL-α-dimyristoyl lecithin	Reactive	Reactive
	Stearoyl glycollecithin	Weakly reactive	Reactive
	L-α-dimyristoyl cephalin		Anticomplementary
	Dipalmitoyl L-α-glycerophosphoric acid monocholine salt	Not reactive	

We have obtained samples of various synthetic lecithins from Baer and have tested them as substitutes for beef-heart lecithin in cardiolipin antigens. Other materials synthesized by Baer and his associates have also been investigated as lecithin substitutes. These are L-α-dimyristoyl cephalin, stearoyl glycollecithin, and dipalmitoyl-L-α-glycerophosphoric acid monocholine salt. In addition, tetramyristoyl-bis-(L-α-glyceryl) phosphoric acid (*3*), a material somewhat similar in

0.0175%; synthetic lecithin, 0.2%; cholesterol, 0.3%. Others have found synthetic lecithins to be reactive. In a preliminary study, Rosenberg (*4*) noted that antigens (Hinton, Kline, Kolmer, Rein-Bossak, V.D.R.L.) prepared with synthetic dipalmitoyl lecithin reacted antigenically. Kline (*5*) has studied the use of dimyristoyl lecithin as an antigen component with cardiolipin and Faure (*6*) has investigated the substitution of dimyristoyl lecithin for natural leci-