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 Unpublished data.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).

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

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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-

thin in the Kline test and both the Kolmer and Debains complement fixation tests.

We have also prepared antigens with D-α-dimyristoyl lecithin and DL-α-dimyristoyl lecithin and have used them in the V.D.R.L. slide test in parallel with L-α-dimyristoyl lecithin antigen of the same composition. Very similar results were obtained. There was also good agreement when the 3 lecithins were used in parallel in the Kolmer test.

Stearoyl glycollecithin was used as a substitute for lecithin in a wide range of concentrations in the V.D.R.L. slide test. With syphilitic sera, flocculation occurred but the various antigens were undersensitive compared with V.D.R.L. slide test antigen. Coarse particles, and often small aggregates of particles, were observed with normal sera. In the Kolmer test a level of sensitivity approximately that of Kolmer antigen was obtained with the following antigen mixture: cardiolipin, 0.03%; glycollecithin, 0.075%; cholesterol, 0.3%. No anticomplementary or hemolytic properties were detected.

Difficulty was encountered in the substitution of L-α-dimyristoyl cephalin for beef-heart lecithin due to the limited solubility of the material in absolute ethyl alcohol. Two antigen mixtures were prepared for use in the Kolmer test and they proved to be anticomplementary.

No serological reactivity was observed when dipalmitoyl-L-α-glycerophosphoric acid monocholine salt was used in place of natural lecithin in V.D.R.L. slide test antigen.

The phosphatidic acid, tetramyristoyl-bis-(L-α-glyceryl) phosphoric acid was substituted for cardiolipin in antigen mixtures prepared for the V.D.R.L. test. Flocculation occurred with strongly positive syphilitic sera. When normal sera were examined, the test mixtures were often coarse and difficult to read. In the Kolmer test, certain phosphatidic acid antigen mixtures showed definite reactivity with positive sera, but even the most reactive (phosphatidic acid, 0.08%; lecithin, 0.033%; cholesterol, 0.3%) was less sensitive than Kolmer lipoidal antigen. Possibly other phosphatidic acids may prove to be more satisfactory. Faure (*7*) has found that unsaturated phosphatidic acids extracted from plants can be substituted for cardiolipin but that the resultant antigen is less reactive.

The use of pure synthetic compounds in the preparation of antigens would offer certain advantages in the standardization of serodiagnostic tests for syphilis. In addition, the investigation holds some promise of revealing the mechanism of reaction and perhaps the molecular groupings which are involved. Further studies are being conducted.

References

1. BAER, E., and KATES, M. *Science*, **109**, 31 (1949); *J. Am. Chem. Soc.*, **72**, 942 (1950).
2. BAER, E., and MAURUKAS, J. *J. Am. Chem. Soc.*, **74**, 158 (1952).
3. BAER, E. *J. Biol. Chem.*, **198**, 853 (1952).
4. ROSENBERG, A. A. *J. Venereal Disease Inform.*, **30**, 194 (1949).
5. KLINE, B. S. *Am. J. Syphilis, Gonorrhea, and Venereal Diseases*, **34**, 460 (1950).
6. FAURE, M. *Ann. Inst. Pasteur*, **82**, 738 (1952).
7. ———. *Bull. soc. chim. biol.*, **31**, 1362 (1949).

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Comments and Communications

Chromatogram Spotting Apparatus[1,2]

THE chromatogram spotting apparatus[3] shown was designed and built in an attempt to standardize the technique of placing liquid material on paper chromatograms in definite amounts. Experience in this laboratory has shown that considerable variability in chromatograms results when several individuals are doing the spotting, as occurs in the case of a paper chromatographic study involving samples of many field replications. This equipment enables the worker to standardize the technique and facilitate ease of operation, thus making it possible for less experienced employees to do uniform, high quality spotting.

The principal error in spotting seems to be due to a failure to place the micropipette at right angles to the paper. This often results in an irregular spot. The parallelogram arm pipette holder eliminates this error. A T-square notched at regular intervals reduces the usual time-consuming process of measuring and marking one-dimensional papers to be spotted. The lighted recess or well in the board directly under the spotting

FIG. 1.

[1] Published with approval of the director, Colorado Agricultural Experiment Station, as G. S. paper No. 525.

[2] This equipment was developed during the course of a biochemical investigation supported in part by the Herman Frasch Foundation.

[3] A complete set of detailed blueprints and photographs may be obtained for $5.00 to cover cost of preparation by writing directly to Clark Livingston, Chemistry Department, Colorado A. & M. College, Fort Collins, Colorado.