VOL. 2 APRIL, 1927 NO. 15

HILGARDIA

A Journal of Agricultural Science

PUBLISHED BY THE

California Agricultural Experiment Station

CONTENTS

UNIVERSITY OF CALIFORNIA PRINTING OFFICE
BERKELEY, CALIFORNIA

THE ELIMINATION OF CLOUDY REACTIONS BY THE USE OF FORMALIN AS A PRESERVATIVE FOR *BACTERIUM PULLORUM* ANTIGEN

J. R. BEACH[1] AND S. TER-MICHAELIAN[2]

INTRODUCTION

All who have used the agglutination test for the detection of fowls that harbor *Bacterium pullorum* have observed the occurrence of excessive turbidity in many tubes which seriously interfered with accurate reading of the reactions. Hitchner,[3] in 1923, reported that the turbidity resulted from the precipitation of fat that is present in the blood serum of some fowls and that it could be avoided by starving fowls for thirty-six hours before blood samples were drawn. Matthews,[4] in 1926, reported studies which he believed demonstrated that such turbidity was due to the presence of a protein rather than a fatty substance in blood serum of fowls. He stated that this protein substance was soluble in weak alkali solution and that clouding of agglutination tests could be avoided by adding a small amount of sodium hydroxide solution to antigen.

Bushnell, Hinshaw and Payne,[5] in 1926, published a very complete discussion of bacillary white diarrhea in fowls which included a résumé of the methods used by various agricultural experiment station laboratories in making agglutination tests. This résumé shows that in twenty-four of twenty-eight laboratories, phenol is used for preservation of the antigen. The amount of phenol used is 0.5 per cent in nineteen laboratories and 0.4, 0.3, 0.25 and 0.2 per cent phenol in one each of four other laboratories. One laboratory was reported as

[1] Assistant Professor of Veterinary Science and Associate Veterinarian in the Experiment Station.

[2] Research Assistant in Veterinary Science.

[3] Hitchner, E. R. The macroscopic agglutination test as influenced by the fatty content of the blood serum of fowls. Jour. Amer. Vet. Med. Assn. 63: 759–763. 1923.

[4] Matthews, F. P. Obscured reactions in the agglutination test for bacillary white diarrhea. Jour. Immunology 11:499–504. 1926.

[5] Bushnell, L. D., W. R. Hinshaw, and L. F. Payne. Bacillary white diarrhea in fowl. Kansas Agr. Exp. Sta. Tech. Bul. 21:1–858, figs. 1–4. 1926.

using either 0.5 per cent phenol or 0.5 per cent formalin; one as using a coal tar disinfectant (no percentage given); and two as using no preservative in the antigen. It is seen from the above that preservation of antigen by the addition of 0.5 per cent phenol is the prevailing practice. In a further discussion of their own technique and that of other laboratories, these writers disclose that the reason for using 0.3 per cent or a lesser amount of phenol in antigen is to avoid confusing turbidity. The writers also state that "Formolized antigens do not cause a precipitation of the fat-like substance but antigens so prepared are not as reliable as when preserved with phenol." Tittsler, in Pennsylvania, however, is reported by them as using 0.5 per cent formalin for preserving antigen.

The laboratory of the Division of Veterinary Science, California Agricultural Experiment Station, was one of those using antigen containing 0.5 per cent phenol in routine testing of breeding flocks. Difficulty in interpreting the results of the tests on account of turbidity produced by certain sera was frequently encountered. An investigation of means of avoiding turbid or cloudy reactions was, therefore, undertaken. This investigation consisted of a comparison of the results of agglutination tests of the same sera with antigens containing varying amounts of phenol or formalin.

Tests with Antigens Containing 0.1, 0.25, or 0.5 Per Cent Phenol

The antigens were prepared by washing off the growth from 48-hour agar cultures of *Bact. pullorum* with a small amount of physiological salt solution containing 0.5 per cent phenol. For the tests, this was diluted with sufficient physiological saline with or without the addition of phenol to give a reading of 3.5 cm. with a Gates[6] nephelometer and to make the final product contain the desired amount of phenol.

Forty-eight sera were tested with three antigens containing 0.1, 0.25 and 0.5 per cent of phenol respectively. Serum-antigen dilutions of 1–25 and 1–50 were used. Readings were made after 24 hours at 37.5° C and 24 hours at room temperature. The results are as follows:

No positive reactions to the test occurred.

In seventeen of the tests with 0.5 per cent phenolized antigen, there was either increased cloudiness of the fluid or sediment at the bottom of the tubes from the precipitation of a substance from the sera.

[6] Gates, F. L. A method of standardizing bacterial suspensions. Jour. Exp. Med. 31:105–114. 1920.

In ten of the tests with the 0.25 per cent phenolized antigen, there was some increase in cloudiness of the fluid due to a substance in the sera. The cloudiness in these cases was not nearly as marked as that which occurred in the tests of the same sera with 0.5 per cent phenolized antigen.

In none of the tests with the 0.1 per cent phenolized antigen was there any cloudiness or sediment due to precipitation of a substance in the serum. In all 48 tests with this antigen, however, there was either an increase in cloudiness or there was sediment at the bottom of the tubes resulting from the multiplication of contaminating organisms that were present in the sera.

The results of these preliminary agglutination tests of fowl serum with phenolized antigens indicated that cloudiness due to the precipitation of a substance in the serum could be lessened in intensity or entirely avoided by using less than 0.5 per cent phenol in the antigen. When 0.25 per cent phenol was used, the degree of cloudiness from this cause was greatly reduced but not entirely eliminated. When 0.1 per cent phenol was used, no cloudiness from the precipitation of substances in the serum occurred. This latter amount of phenol, however, was insufficient to prevent the multiplication of the contaminating organisms in the serum and, therefore, would be unsatisfactory for use unless the blood was drawn and the test carried out under aseptic conditions.

Tests with Antigens Containing 0.5 Per Cent Phenol, 0.5 Phenol, 0.5 Per Cent Formalin or 0.1 Per Cent Formalin

The 0.5 per cent phenolized antigen and 0.5 per cent formolized antigen were prepared by washing 48-hour agar cultures with a small amount of 0.5 per cent phenolized or formolized physiological salt solution and diluting this concentrated suspension sufficiently with 0.5 per cent phenolized or formolized saline solution at the time of use. The 0.1 per cent formolized antigen was prepared by diluting the concentrated 0.5 per cent formolized antigen with physiological salt solution and 0.1 per cent formolized physiological salt solution at the time of use. The turbidity standard of all antigens was a 3.5 cm. reading with a Gates' nephelometer.

Agglutination tests of 970 sera were made with each of the three antigens. The dilutions of serum and antigen used were 1–25 and 1–50. The results are as follows:

In no case was there any cloudiness or sediment resulting from bacterial multiplication.

Cloudiness occurred in 340, or 35.0 per cent of the tests with phenolized antigen. No cloudiness occurred in the tests with formolized antigens.

A positive reaction in the 0.1 per cent formolized antigen was obtained with 168, or 17.3 per cent of the sera. Of these 168 sera that gave a positive reaction with the 0.1 per cent formolized antigen, 127 sera (or 13.0 per cent of the total sera) also reacted positively with the other two antigens; 13 sera (1.3 per cent of the total sera) also reacted positively with the 0.5 per cent phenolized antigen but not with the 0.5 per cent formolized antigen; 19 sera (1.9 per cent of the total sera) also gave a positive reaction with the 0.5 per cent formolized antigen but not with the phenolized antigen; and 9 sera (0.9 per cent of the total sera) did not react with either of the other two antigens.

All sera that reacted with either the phenolized or 0.5 per cent formolized antigen also reacted with the 0.1 per cent formolized antigen.

Twenty-two of the twenty-eight sera that gave a positive agglutination with the 0.1 per cent formolized antigen and no recognizable agglutination with the phenolized antigen caused clouding of the phenolized antigen. It is possible, therefore, that these twenty-two sera did cause an agglutination of the phenolized antigen which was obscured by the cloudiness. This may account for much of the discrepancy in the results obtained in these tests with the phenolized and 0.1 per cent formolized antigens.

The explanation of the failure of twenty-two of the sera that reacted with the 0.1 per cent formolized antigen to react with the 0.5 per cent formolized antigen, however, is not so apparent. Since the only variable factor was the amount of formalin in the antigens, it seems possible that, in these instances, the 0.5 per cent formalin may have exerted an unfavorable influence on the agglutination of the organisms in the antigen.

The results of these comparative agglutination tests suggested that formalized antigens are more suitable for tests of fowl serum than phenolized antigens. Of the two amounts of formalin used in antigen, i.e., 0.1 per cent and 0.5 per cent, the former seemed more satisfactory. Therefore, additional comparative tests of fowl sera with 0.5 per cent phenolized antigen and 0.1 per cent formolized antigen were carried out.

Tests with Antigens Containing 0.5 Per Cent Phenol or 0.1 Per Cent Formalin

These tests were carried out as opportunity was afforded between February 23 and December 30, 1926, with blood samples from thirty-four flocks.

The methods of preparation and standardization of the antigens were the same as in the preceding tests. Four serum-antigen dilutions, 1–25, 1–50, 1–100, and 1–200, were used in approximately one-third of the tests and two dilutions, 1–25 and 1–50, in the remainder.

Duplicate tests of 4322 sera with two antigens containing 0.5 per cent phenol and 0.1 per cent formalin, respectively, were made. The results are given in table 1.

As shown in table 1, the number of the 4322 sera that reacted with either one or both of the 0.5 per cent phenolized antigen and the 0.1 per cent formolized antigen was 1009 or 23.3 per cent. Of this number, 83 did not react with the phenolized antigen and 41 did not react with the formolized antigen, leaving 885 (20.4 per cent of all tests or 87.7 per cent of all positive tests) that reacted with both antigens.

Cloudiness of the phenolized antigen was caused by 1700 or 39.3 per cent of the sera. The formolized antigen was not affected. The agglutination reaction of 298 of these sera was recorded as positive with both antigens, of 34 as positive with phenolized antigen only, and of 64 as positive with formolized antigen only. By comparing these numbers with the total number of sera that caused agglutination reaction with only one antigen, it is seen that 34 of 41 sera that gave a reaction recorded as positive with phenolized antigen only, and 64 of 83 sera that gave a reaction recorded as positive with formolized antigen only also caused cloudiness of the phenolized antigen.

Since cloudiness of serum-antigen mixtures makes interpretation of agglutination reactions uncertain, it is possible that incorrect readings were made of many or all of the reactions with the phenolized antigen of those sera that caused cloudiness of the phenolized antigen and an agglutination reaction recorded as positive with one antigen only. In such a case, an incorrect interpretation of the agglutination-test reactions with phenolized antigen may have been made of 34 of the 41 sera that were recorded as reacting with phenolized antigen only and of 64 of the 83 sera that were recorded as reacting with formolized antigen only. This would leave but 26 or 0.6 per cent of all tests in which failure to secure the same interpretation of the agglutination reactions with both antigens might not have been due to the real reaction with the phenolized antigen being obscured by cloudiness.

TABLE 1

RESULTS OF AGGLUTINATION TESTS OF 4,322 FOWL SERA WITH 0.5 PER CENT PHENOLIZED ANTIGEN AND 0.1 PER CENT FORMOLIZED ANTIGEN

Number of sera	Number that reacted				Number cloudy with phenolized antigen			
	With both antigens	With phenolized antigen only	With formolized antigen only	Total	Total	Also reacted with both antigens	Also reacted with phenolized antigen only	Also reacted with formolized antigen only
200	3	0	5	8	151	0	0	4
65	0	0	0	0	10	0	0	0
56	3	0	0	3	46	0	0	0
92	10	0	1	11	27	1	0	0
42	1	0	0	1	25	1	0	0
11	0	0	0	0	0	0	0	0
9	1	0	0	1	1	0	0	0
9	2	0	0	2	0	0	0	0
171	54	0	2	56	52	10	0	2
54	9	0	0	9	3	0	0	0
176	34	0	10	44	61	9	0	6
170	27	0	16	43	87	12	0	14
215	0	0	1	1	56	0	0	1
218	38	0	0	38	49	2	0	0
164	24	0	5	29	86	6	0	5
125	43	0	4	47	11	4	0	4
203	21	0	0	21	103	1	0	0
190	48	1	3	52	63	1	0	2
161	34	0	2	36	68	5	0	1
70	0	0	0	0	22	0	0	0
114	27	0	0	27	21	0	0	0
199	67	0	0	67	64	2	0	0
50	23	0	0	23	7	0	0	0
134	13	0	2	15	48	4	0	0
96	3	0	0	3	62	0	0	0
131	2	0	0	2	82	0	0	0
13	2	0	0	2	0	0	0	0
33	5	0	0	5	0	0	0	0
21	4	0	0	4	12	0	0	0
6	0	0	0	0	0	0	0	0
36	6	0	0	6	10	0	0	0
36	6	0	0	6	0	0	0	0
156	29	0	1	30	31	6	0	1
313	167	30	10	207	275	164	28	8
152	21	2	0	23	16	0	0	0
151	21	0	4	25	1	0	0	1
280	137	8	17	162	150	70	6	15
4322	885	41	83	1009	1700	298	34	64

Seven of the fowls whose blood serum had agglutinated the formolized antigen and had neither clouded nor agglutinated the phenolized antigen were secured for autopsy. The postmortem and bacteriological examinations of two of these birds were negative. Four birds had abnormal yolks in the ovaries. *Bact. pullorum* was isolated from three of these. The seventh bird exhibited no ovarian abnormalities, but a mass of fibrinous exudate was present in the pericardial sac. *Bact. pullorum* was isolated from the exudate. These results demonstrate that at least a part of the fowls that reacted with the formolized antigen only were carriers of *Bact. pullorum.*

Variations in the Degree of the Reactions with the Two Antigens

The preceding discussion of the comparative results of the agglutination tests with 0.5 per cent phenolized and 0.1 per cent formolized antigens has shown that 885 or 20.4 per cent of the sera gave a positive reaction with both antigens. In these tests, partial or complete agglutination in any serum-antigen dilution was considered a positive reaction. This classification, therefore, serves to differentiate the sera which produced no agglutination of either one or both antigens from those which produced some agglutination in one or more dilutions with each antigen, but does not indicate whether agglutination occurred in one or more dilutions or whether the number of dilutions in which agglutination occurred was the same for both antigens. In making an accurate comparison of the results with the two antigens, however, consideration of the degree of agglutination obtained with each antigen must be given. A summary of the data on this point is, therefore, included in this paper.

Two dilutions, i.e., 1–25 and 1–50, were used in 3021 tests and four dilutions, i.e., 1–25, 1–50, 1–100 and 1–200 in 1301 tests. The results are as follows:

Two-dilution Tests.—A positive reaction with both antigens was obtained in 641 tests. In 546, or 85.1 per cent, agglutination occurred in the same dilutions of both antigens as follows:

35 sera agglutinated the 1–25 dilution only.
511 sera agglutinated both the 1–25 and 1–50 dilutions.

In 95, or 14.8 per cent, of the positive tests, agglutination did not occur in the same dilutions of both antigens. The variations in these agglutination reactions were:

40 sera agglutinated the 1–25 dilution only of phenolized antigen and both dilutions of formolized antigen.
55 sera agglutinated both dilutions of phenolized antigen and the 1–25 dilution only of formolized antigen.

There was cloudiness of the phenolized antigen in 57 of the 95 tests. This might have been responsible for much of the difference in the readings of the reactions with the two antigens in these tests.

Four-dilution Tests.—A positive reaction in both antigens was obtained in 244 tests.

In 126, or 51.6 per cent, agglutination occurred in the same dilutions of both antigens as follows:

27 sera agglutinated the 1–25 dilution only.
39 sera agglutinated the 1–25 and 1–50 dilutions only.
8 sera agglutinated the 1–25, 1–50 and 1–100 dilutions.
59 sera agglutinated the 1–25, 1–50, 1–100 and 1–200 dilutions.

In 118, or 48.3 per cent, of the positive tests, the dilutions in which agglutination occurred were not the same for both antigens. The agglutination titre in fourteen of these tests was higher with the phenolized than with the formolized antigen, and in 104 tests the titre was higher with the formolized than with the phenolized antigen. The variations in these agglutination reactions are given in table 2.

TABLE 2

VARIATION IN THE DILUTIONS OF PHENOLIZED AND FORMOLIZED ANTIGENS AGGLUTINATED BY THE SAME SERA. DILUTIONS OF 1–25, 1–50, 1–100 AND 1–200 WERE USED

Number of sera	Dilutions of phenolized antigen agglutinated	Dilutions of formolized antigen agglutinated	Number of sera causing clouding of phenolized antigen
2	1–25, 1–50, 1–100, 1–200	1–25, 1–50	0
3	1–25, 1–50, 1–100, 1–200	1–25, 1–50, 1–100	1
4	1–25, 1–50, 1–100	1–25, 1–50	1
5	1–25, 1–50	1–25	1
6	1–25	1–25, 1–50, 1–100, 1–200	3
28	1–25, 1–50	1–25, 1–50, 1–100, 1–200	6
28	1–25, 1–50, 1–100	1–25, 1–50, 1–100, 1–200	3
14	1–25, 1–50	1–25, 1–50, 1–100	3
5	1–25	1–25, 1–50, 1–100	0
23	1–25	1–25, 1–50	9

It can be seen from the preceding data that there was little difference in the agglutination of the phenolized and formolized antigens in the 1–25 and 1–50 dilutions. Much of the difference that did exist might have been due to incorrect reading of the reaction in the phenolized antigen because of clouding of that antigen by some of the sera. A considerably larger number of sera, however, caused agglutination of the 1–100 and 1-200 dilutions of formolized antigen than in the corresponding dilutions of phenolized antigen.

Discussion

The results of the 4322 comparative tests indicate that antigen containing 0.1 per cent formalin is satisfactory for making agglutination tests of blood serum from fowls. There was little difference in either the number or distribution of the sera which reacted with the two antigens. In the tests in which four dilutions were used and in which reactions to both antigens in at least one dilution were obtained, more sera caused agglutination in the 1–100 and 1–200 dilutions of formolized antigen than in the corresponding dilutions of phenolized antigen.

The cloudiness which occurred in 1700 tests with phenolized antigen did not appear in the corresponding tests with formolized antigen. In this respect, the formolized antigen was more satisfactory than the phenolized antigen.

It was observed that the clumps of bacteria formed by the agglutination of the organisms in the formolized antigen were smaller and more easily broken up than the clumps of bacteria in the phenolized antigen. This was of no importance when complete agglutination occurred, but did make the reading of partial agglutinations more difficult in the formolized antigen than in the phenolized antigen. This feature of the behavior of formolized antigen, however, is an unimportant source of error in the interpretation of agglutination reactions when compared with the frequently-occurring cloudiness of phenolized antigen.

PUBLICATIONS AVAILABLE FOR FREE DISTRIBUTION

BULLETINS

No.

253. Irrigation and Soil Conditions in the Sierra Nevada Foothills, California.
261. Melaxuma of the Walnut, "Juglans regia."
262. Citrus Diseases of Florida and Cuba Compared with Those of California.
263. Size Grades for Ripe Olives.
268. Growing and Grafting Olive Seedlings.
273. Preliminary Report on Kearney Vineyard Experimental Drain.
275. The Cultivation of Belladonna in California.
276. The Pomegranate.
277. Sudan Grass.
278. Grain Sorghums.
279. Irrigation of Rice in California.
283. The Olive Insects of California.
294. Bean Culture in California.
304. A Study of the Effects of Freezes on Citrus in California.
310. Plum Pollination.
312. Mariout Barley.
313. Pruning Young Deciduous Fruit Trees.
319. Caprifigs and Caprification.
324. Storage of Perishable Fruit at Freezing Temperatures.
325. Rice Irrigation Measurements and Experiments in Sacramento Valley, 1914–1919.
328. Prune Growing in California.
331. Phylloxera-Resistant Stocks.
335. Cocoanut Meal as a Feed for Dairy Cows and Other Livestock.
339. The Relative Cost of Making Logs from Small and Large Timber.
340. Control of the Pocket Gopher in California.
343. Cheese Pests and Their Control.
344. Cold Storage as an Aid to the Marketing of Plums.
346. Almond Pollination.
347. The Control of Red Spiders in Deciduous Orchards.
348. Pruning Young Olive Trees.
349. A Study of Sidedraft and Tractor Hitches.
350. Agriculture in Cut-over Redwood Lands.
352. Further Experiments in Plum Pollination.
353. Bovine Infectious Abortion.
354. Results of Rice Experiments in 1922.
357. A Self-mixing Dusting Machine for Applying Dry Insecticides and Fungicides.
358. Black Measles, Water Berries, and Related Vine Troubles.
361. Preliminary Yield Tables for Second Growth Redwood.
362. Dust and the Tractor Engine.
363. The Pruning of Citrus Trees in California.
364. Fungicidal Dusts for the Control of Bunt.
365. Avocado Culture in California.
366. Turkish Tobacco Culture, Curing and Marketing.
367. Methods of Harvesting and Irrigation in Relation of Mouldy Walnuts.
368. Bacterial Decomposition of Olives during Pickling.
369. Comparison of Woods for Butter Boxes.

No.

370. Browning of Yellow Newtown Apples.
371. The Relative Cost of Yarding Small and Large Timber.
372. The Cost of Producing Market Milk and Butterfat on 246 California Dairies.
373. Pear Pollination.
374. A Survey of Orchard Practices in the Citrus Industry of Southern California.
375. Results of Rice Experiments at Cortena, 1923.
376. Sun-Drying and Dehydration of Walnuts.
377. The Cold Storage of Pears.
379. Walnut Culture in California.
380. Growth of Eucalyptus in California Plantations.
381. Growing and Handling Asparagus Crowns.
382. Pumping for Drainage in the San Joaquin Valley, California.
383. Monilia Blossom Blight (Brown Rot) of Apricot.
385. Pollination of the Sweet Cherry.
386. Pruning Bearing Deciduous Fruit Trees.
387. Fig Smut.
388. The Principles and Practice of Sun-drying Fruit.
389. Berseem or Egyptian Clover.
390. Harvesting and Packing Grapes in California.
391. Machines for Coating Seed Wheat with Copper Carbonate Dust.
392. Fruit Juice Concentrates.
393. Crop Sequences at Davis.
394. Cereal Hay Production in California. Feeding Trials with Cereal Hay.
395. Bark Diseases of Citrus Trees.
396. The Mat Bean (Phaseolus aconitifolius).
397. Manufacture of Roquefort Type Cheese from Goat's Milk.
398. Orchard Heating in California.
399. The Blackberry Mite, the Cause of Redberry Disease of the Himalaya Blackberry, and its Control.
400. The Utilization of Surplus Plums.
401. Cost of Work Horses on California Farms.
402. The Codling Moth in Walnuts.
403. Farm-Accounting Associations.
404. The Dehydration of Prunes.
405. Citrus Culture in Central California.
406. Stationary Spray Plants in California.
407. Yield, Stand and Volume Tables for White Fir in the California Pine Region.
408. Alternaria Rot of Lemons.
409. The Digestibility of Certain Fruit By-products as Determined for Ruminants.
410. Factors Affecting the Quality of Fresh Asparagus after it is Harvested.
411. Paradichlorobenzene as a Soil Fumigant.
412. A Study of the Relative Values of Certain Root Crops and Salmon Oil as Sources of Vitamin A for Poultry.
413. The California Poultry Industry; a Statistical Study.
414. Planting and Thinning Distances for Deciduous Fruit Trees.

CIRCULARS

No.
87. Alfalfa.
117. The Selection and Cost of a Small Pumping Plant.
127. House Fumigation.
129. The Control of Citrus Insects.
136. *Melilotus indica* as a Green-Manure Crop for California.
144. Oidium or Powdery Mildew of the Vine.
157. Control of the Pear Scab.
160. Lettuce Growing in California.
164. Small Fruit Culture in California.
166. The County Farm Bureau.
170. Fertilizing California Soils for the 1918 Crop.
173. The Construction of the Wood-Hoop Silo.
178. The Packing of Apples in California.
179. Factors of Importance in Producing Milk of Low Bacterial Count.
190. Agriculture Clubs in California.
199. Onion Growing in California.
202. County Organizations for Rural Fire Control.
203. Peat as a Manure Substitute.
209. The Function of the Farm Bureau.
210. Suggestions to the Settler in California.
212. Salvaging Rain-Damaged Prunes.
215. Feeding Dairy Cows in California.
217. Methods for Marketing Vegetables in California.
220. Unfermented Fruit Juices.
228. Vineyard Irrigation in Arid Climates.
230. Testing Milk, Cream, and Skim Milk for Butterfat.
231. The Home Vineyard.
232. Harvesting and Handling California Cherries for Eastern Shipment.
234. Winter Injury to Young Walnut Trees during 1921–22.
235. Soil Analysis and Soil and Plant Inter-relations.
236. The Common Hawks and Owls of California from the Standpoint of the Rancher.
237. Directions for the Tanning and Dressing of Furs.
238. The Apricot in California.
239. Harvesting and Handling Apricots and Plums for Eastern Shipment.
240. Harvesting and Handling Pears for Eastern Shipment.
241. Harvesting and Handling Peaches for Eastern Shipment.
243. Marmalade Juice and Jelly Juice from Citrus Fruits.
244 Central Wire Bracing for Fruit Trees.
245. Vine Pruning Systems.
247. Colonization and Rural Development.
248. Some Common Errors in Vine Pruning and Their Remedies.
249. Replacing Missing Vines.
250. Measurement of Irrigation Water on the Farm.
252. Supports for Vines.
253. Vineyard Plans.
254. The Use of Artificial Light to Increase Winter Egg Production.

No.
255. Leguminous Plants as Organic Fertilizer in California Agriculture.
256. The Control of Wild Morning Glory.
257. The Small-Seeded Horse Bean.
258. Thinning Deciduous Fruits.
259. Pear By-products.
261. Sewing Grain Sacks.
262. Cabbage Growing in California.
263. Tomato Production in California.
264. Preliminary Essentials to Bovine Tuberculosis Control.
265. Plant Disease and Pest Control.
266. Analyzing the Citrus Orchard by Means of Simple Tree Records.
267. The Tendency of Tractors to Rise in Front: Causes and Remedies.
269. An Orchard Brush Burner.
270. A Farm Septic Tank.
272. California Farm Tenancy and Methods of Leasing.
273. Saving the Gophered Citrus Tree.
274. Fusarium Wilt of Tomato and its Control by Means of Resistant Varieties.
276. Home Canning.
277. Head, Cane, and Cordon Pruning of Vines.
278. Olive Pickling in Mediterranean Countries.
279. The Preparation and Refining of Olive Oil in Southern Europe.
281. The Results of a Survey to Determine the Cost of Producing Beef in California.
282. Prevention of Insect Attack on Stored Grain.
283. Fertilizing Citrus Trees in California.
284. The Almond in California.
285. Sweet Potato Production in California.
286. Milk Houses for California Dairies.
287. Potato Production in California.
288. Phylloxera Resistant Vineyards.
289. Oak Fungus in Orchard Trees.
290. The Tangier Pea.
291. Blackhead and Other Causes of Loss of Turkeys in California.
292. Alkali Soils.
293. The Basis of Grape Standardization.
294. Propagation of Deciduous Fruits.
295. The Growing and Handling of Head Lettuce in California.
296. Control of the California Ground Squirrel.
298. The Possibilities and Limitations of Coöperative Marketing.
299. Poultry Breeding Records.
300. Coccidiosis of Chickens.
301. Buckeye Poisoning of the Honey Bee.
302. The Sugar Beet in California.
303. A Promising Remedy for Black Measles of the Vine.
304. Drainage on the Farm.
305. Liming the Soil.
306. A General Purpose Soil Auger and its Use on the Farm.
307. American Foulbrood and its Control.
308. Cantaloupe Production in California.

The publications listed above may be had by addressing

College of Agriculture,
University of California,
Berkeley, California.

The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.
3. The Formation of Sodium Carbonate in Soils, by Arthur B. Cummins and Walter P. Kelley. March, 1923.
4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.
5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.
9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.
12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.
13. Some Mutual Effects on Soil and Plant Induced by Added Solutes, by John S. Burd and J. C. Martin. December, 1923.
14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.
15. Replaceable Bases in Soils, by Walter P. Kelley and S. Melvin Brown. February, 1924.
16. The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.
17. Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.
18. Factors Influencing the Rate of Germination of Seed of Asparagus officinalis, by H. A. Borthwick. March, 1925.
19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.
20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.