College Station on December 4, 1934, was informal and abstracts of results were not published. After an interval of three years, the seventh conference, September 21–24, 1937, took the form of a tour of field experiments from Greenville to College Station, with informal meetings along the line of travel. The 1940 conference, making the eighth of the series, took place again after an interval of three years and was also a tour of field stations and plot experiments with sessions at points along the route for discussion of field and laboratory results.

The tour began with inspection of work at the U. S. Cotton Field Station at Greenville, during the morning of August 6. The party then proceeded to Temple, via Rosebud, where soil fertility plot experiments were seen. The morning of August 7 was devoted to a rapid examination of extensive root-rot work at Substation No. 5 of the Texas Agricultural Experiment Station, near Temple. Field plots of the Clayton Foundation of the University of Texas were seen that afternoon in the vicinity of Austin, and laboratories at the university were visited during the evening. On August 8, soil fertility plots were visited at Kimbro and Elgin; and plot experiments at Brenham were viewed en route to College Station. The program concluded with an informal session at College Station on August 9, during which experimental work not visited on the tour was briefly summarized and discussed.

As usual, attendance at this meeting included most of the workers engaged in study of cotton root-rot, along with the heads of the organizations concerned. Omitting visitors, the 41 attending may be classified as: plant pathologists 9, agronomists 7, plant physiologists 4, soil bacteriologists 4, agricultural aides 4, chemists 3, geneticists 3, substation superintendents 3, soil technologists 2, entomologists 1 and botanists 1. Research groups represented were the University of Arizona Agricultural Experiment Station; the U. S. Department of Agriculture Cotton Field Stations at Sacaton, Arizona, and at Greenville, Texas; the U. S. D. A. Divisions of Cotton and Other Fiber Crops and Diseases, Soil Microbiology, and Cereal Crops and Diseases; the U. S. D. A. Soil Fertility Laboratory at Austin; the Clayton Foundation of the University of Texas; the A. and M. College of Texas; and the Texas Agricultural Experiment Station at College Station and Substations No. 5 at Temple and No. 15 at Weslaco.

No prepared papers were presented at the sessions of this meeting and results of the work were given informally. Publication of abstracts of these results is therefore not contemplated.

WALTER N. EZEKIEL
DIVISION OF PLANT PATHOLOGY AND PHYSIOLOGY,
TEXAS AGRICULTURAL EXPERIMENT STATION,
A. AND M. COLLEGE OF TEXAS

SPECIAL ARTICLES

A QUANTITATIVE, ABSOLUTE METHOD FOR THE ESTIMATION OF COMPLEMENT (ALEXIN)

THE quantitative absolute methods introduced by this laboratory[1] for the study of specific immune precipitation and agglutination have now been extended to the estimation of complement (alexin). If, as defined by Muir,[2] complement is "that labile substance of normal serum which is taken up by the combination of an antigen and its anti-substance (immune body)," then complement may be measured in milligrams of nitrogen per milliliter as outlined below.

Quantitative precipitin estimations were run with proportions of antigen and rabbit antisera such that not quite all of the antibody was precipitated. In this way disc-like precipitates were avoided, as these are difficult to disintegrate and wash thoroughly. All guinea-pig serum used was neutralized to phenol red in the hope of reducing the solubility of the specific precipitates. The rabbit sera (inactivated) contained sufficient antibody nitrogen for quantitative estimations at dilutions which were not anticomplementary. 1.0 ml portions of antiserum dilution were added, each in triplicate, to 5.0 ml of 0.9 per cent. saline, 5.0 ml of heat-inactivated guinea-pig serum and 5.0 ml samples of an unheated portion of the same guinea-pig serum pool. The tube contents were mixed and 1.0 ml of antigen dilution was added to each tube and mixed. Blank tubes were also set up with active and inactivated complement to which antigen or antiserum alone was added. After 1 hour at room temperature the tubes were centrifuged in the cold and the analyses were completed in the usual way,[3] except that in order to assure maximum accuracy all supernatants were recentrifuged as in the agglutination procedure[4] and all tubes were washed three times with chilled saline instead of twice, owing to the large amounts of guinea-pig serum used. Washings were tested and found free from complement. The hemolytic unit of complement activity was measured in the usual way with 0.2 ml of minimally sensitized sheep red-cell suspension (2 to 2.5

[1] Reviewed in *Chem. Revs.*, 24: 323, 1939; *Bact. Revs.*, 3: 49, 1939.

[2] R. Muir, "Studies on Immunity," Oxford University Press, London, 1909.

[3] M. Heidelberger and F. E. Kendall, *Jour. Exp. Med.*, 61: 559, 1935; 62: 697.

[4] M. Heidelberger and E. A. Kabat, *Jour. Exp. Med.*, 67: 545, 1938.

per cent. final concentration). Hemolytic units in the supernatants from the tubes which had contained active complement were estimated from the largest non-anticomplementary volume failing to show appreciable hemolysis, and were therefore actually much less than the number indicated, except in the second experiment, in which complete hemolysis was actually obtained at the level given. The results are summarized in Table 1.

TABLE 1

Precipitating system, rabbit serum	Hemolytic units of complement		Nitrogen precipitated				
	Added	Left in supernatant	Antigen, serum, saline	Antigen, serum, heat-inactivated complement	Antigen, serum, complement	Difference between active and inactivated complement	N precipitated per 1,000 hemolytic units of complement
			mg	*mg*	*mg*	*mg*	*mg*
Pn III*	1,250	<10	0.588	0.598	0.720	0.122	0.10
" "†	1,250	40	0.388	0.406	0.562	0.156	0.13
Ea-anti-Ea‡	1,000	<<75	0.478	0.484	0.604	0.120	0.12

* Antipneumococcus Type III serum, specific polysaccharide of Type III pneumococcus (S III).

† In this experiment and the one following the entire guinea-pig serum pool was filtered through Gradocol membranes of 700 mμ average pore diameter.

‡ Anti-egg albumin serum, crystalline egg albumin. In this experiment a Type I antipneumococcus horse specific precipitate, known not to fix complement (H. Zinsser and J. T. Parker, *Jour. Immunol.*, 8: 151, 1923; K. Goodner and F. L. Horsfall, Jr., *Jour. Exp. Med.*, 64: 201, 1936) was first formed in the active and inactivated complement used. Identical amounts of nitrogen (0.437, 0.437 mg per 5.0 ml) were precipitated (0.113 mg. N from the saline-SI-anti-PnI blank), but the antipneumococcus horse serum action of the horse serum used reduced the hemolytic units by one third. The value given in the table is that originally found.

It would appear, therefore, that roughly 1,000 hemolytic units, as measured above, correspond to about 0.12 mg of complement N, as defined above. If complement is actually a globulin, as now seems established,[5] this would correspond to about 0.75 mg of protein and indicate that 1 ml of guinea-pig serum ordinarily contained about 0.024 to 0.03 mg of complement N, or 0.15 to 0.20 mg of actual complement. Additional computations which follow directly from this analytical result and a discussion of some of the implications of these findings, which are being extended in several directions, will be given in a more detailed paper now in preparation.

MICHAEL HEIDELBERGER
DEPARTMENT OF MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE PRESBYTERIAN HOSPITAL, NEW YORK

[5] T. W. B. Osborne, "Complement or Alexin," Oxford University Press, 1937; E. E. Ecker, L. Pillemer, C. B. Jones and S. Seifter, *Jour. Biol. Chem.*, 135: 347, 1940.

THE THERAPEUTIC EFFECTIVENESS OF A PRACTICALLY NONTOXIC NEW COMPOUND (CALCIUM AUROTHIOMALATE) IN EXPERIMENTAL, PROLIFERATIVE, CHRONIC ARTHRITIS OF MICE

EARLY in 1939, one of us (A. B. S.) reported that it was possible by intravenous injection of a newly discovered pleuropneumonia-like microorganism to produce an experimental, proliferative, chronic arthritis in mice which clinically and pathologically bears a close resemblance to human rheumatoid arthritis.[1] Although repeated attempts have failed to reveal the presence of such a microorganism in the human disease,[2,3,4] it was found that the experimental arthritis of mice responded to certain chemotherapeutic agents in a manner paralleling their alleged effectiveness or ineffectiveness in rheumatoid arthritis.[5] Thus, of many substances tested, the inorganic and organic gold compounds were the only ones capable of exerting a curative effect on the experimental arthritis in mice, despite the fact that they had no demonstrable action on the etiological agent *in vitro*. It was also found that the toxicity, as measured by the lethal effect on mice, and therapeutic effectiveness were a function of different properties of the gold compounds, and that depending upon their structure and mode of administration there was a wide range in the margin of safety as reflected in chemotherapeutic indexes which varied from 2 to over 30.[5] The present study obtained its orientation from the observation that while colloidal preparations of gold or of gold sulfide were therapeutically inert, a distinct, though delayed, curative effect followed the administration of large doses of an insoluble gold compound, calcium aurothioglycolate, of which mice tolerated an amount at least ten times as large as the minimal therapeutic dose.[5]

The purpose of this communication is to report that it has proved possible to prepare a compound with practically no toxicity and even greater therapeutic effectiveness simply by converting sodium aurothiomalate[6] into calcium aurothiomalate. The addition of an excess of $CaCl_2$ to a solution of sodium aurothiomalate leads to practically complete precipitation of a com-

[1] A. B. Sabin, SCIENCE, 89: 228, 1939.
[2] *Ibid.*, SCIENCE, 90: 18, 1939.
[3] A. B. Sabin and B. Johnson, *Proc. Soc. Exp. Biol. and Med.*, 44: 565, 1940.
[4] G. M. Findlay, R. D. Mackenzie and F. O. MacCallum, *Brit. Jour. Exp. Path.*, 21: 13, 1940.
[5] A. B. Sabin and J. Warren, *Jour. Bact.*, 40: 823, 1940 (in press); also unpublished observations.
[6] We are grateful to Merck and Company for supplying the sodium aurothiomalate (myochrysine) used in this investigation.