ting these projects under way as readily as possible will be the dissemination of the information relative to all phases of electronic development to radio engineers. The fact that the art is highly specialized together with the security rules that have been in force have narrowed down the activities in each individual's field to but a few specialists. But the number of these projects is large and a far-reaching guided program of cooperation is needed to make such developments of greatest utility to a world-wide public. This matter has concerned the industry itself to no small degree.

The promotion of engineering standards will provide all engineers with a common set of terms and test methods. The work of arranging the preparation and publishing of the details of hundreds of application principles (many of them now classified as military secrets) is another phase of this work. Giving greater service to engineers in more remote sections through enlarged and more numerous branch sections is a third. Providing information service to those engineers of the required quality in such a highly technical and widely diversified field requires not only additions to the staff of men having the specific qualities in training and experience to handle the jobs, but also adequate equipment facilities so that their help can be most effectively utilized. All in all, the plans include the following activities: publication of important papers with less delay, post-war publication of a large amount of material now held secret, more adequate publication coverage of the subdivisions of radio-and-electronic interest, publication of a handbook, annual publication of a Year Book, better correspondence service with the sections, program aid to sections, section aid by traveling lectureships, formation and professional direction of semi-autonomous specialist groups in the larger sections, integration of a conference program reaching all parts of the country, proper organization of college activities and other educational work, full-time supervision of standardization activity, creation and housing of a technical library, establishment of employment and placement bureau, activity in legislative matters, additional liaison work with other societies, Government and engineering bodies.

The nucleus of this program will be the concentration of all activities undertaken by the enlarged institute, in a new headquarters building, which will become a center for the promotion of all related activities. In view of the most remarkable strides that have been made by the engineers of this organization in giving to this war the most scientific devices ever developed by any country, this building will have international importance, because the peace-time products of the same group will have world-wide value.

Already the impact of this project has been shown by the nationwide response of both the members and the industrial organizations who foresee the significance of the work, in providing the financial backing of such a headquarters building to insure adequate handling of the needed activities. The response is also remarkable as to the number of new applications for membership from the engineers and research workers in this profession who see in this program the most practical way of getting "educated" as to all the advances made by others during the past five years.

In this expansion, the institute will have the same democratic status as it has had during more than thirty years of its existence. It is the broad-minded cooperation by the members themselves that has heretofore made the publication of all important developments possible, and the work of administering the affairs has always been done by officers who have been elected from those practicing the profession and knowing its problems.

The enthusiasm evident from the response to the call for help on this building project has been such that assurance can be given that it will go through, and work is progressing to the next steps in the plan, that of the procurement and outfitting a suitable structure. The directors are reserving the right to join with other engineering and scientific societies in a common building program if that seems advantageous.

SPECIAL ARTICLES

THE PRECIPITATION OF PURIFIED CONCENTRATED INFLUENZA VIRUS AND VACCINE ON CALCIUM PHOSPHATE[1]

DURING a study[2] of methods useful in the concentration and purification of influenza virus, data on a method involving the precipitation of virus on calcium phosphate[3] were not presented because in preliminary experiments effective purification and concentration of virus were not achieved and because the method appeared impractical for the preparation of virus on a large scale. However in view of the finding by Salk[4] that the precipitation on calcium phos-

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.

[2] W. M. Stanley, *Jour. Exp. Med.*, 79: 255, 1944.

[3] J. E. Salk, *Proc. Soc. Exp. Biol. and Med.*, 46: 709, 1941.

phate of formalinized influenza virus from allantoic fluid yields a product possessing an enhanced immunizing potency, it appeared desirable to present data on the nature of this precipitate. It also appeared desirable to study the precipitation of purified, concentrated influenza virus[5, 6] and vaccine[7] on calcium phosphate with a view to the use of suspensions of such preparations for purposes of immunization.

In order to establish the amounts of reagents necessary to insure precipitation of essentially all the virus in allantoic fluids, increasing amounts of molar solutions of sodium phosphate, calcium chloride and sodium hydroxide were added to five 100 cc portions of freshly harvested PR8 allantoic fluid. The sodium phosphate buffer at pH 8 was added first to insure the presence of sufficient phosphate ions. The calcium chloride and sodium hydroxide were added simultaneously, dropwise and with stirring. The precipitates were removed by centrifugation in a clinical centrifuge and in order to prevent the mechanical transfer of any of the precipitates the supernatant liquids were decanted through filter paper. The amounts of the reagents used and the analyses on the supernatant fluids are given in Table 1. The

TABLE 1

AMOUNTS OF CHICKEN RED CELL AGGLUTINATING (CCA) ACTIVITY AND NITROGEN PRECIPITATED FROM INFECTIOUS PR8 ALLANTOIC FLUID ON ADDITION OF DIFFERENT AMOUNTS OF CALCIUM CHLORIDE

Volume of reagents used*				Supernatant liquid†				
							Test with	
PR8 allantoic fluid	1 M sodium phosphate pH 8	1 M calcium chloride	1 M sodium hydroxide	CCA activity	Total N	pH	Calcium ions‡	Phosphate ions
cc	cc	cc	cc	units/cc	mg/cc			
100	0.00	0.0	0.0	250	0.68	8.1	+	−
100	0.25	0.5	0.4	197	0.65	8.1	+	−
100	0.50	1.0	0.8	105	0.62	8.3	+	−
100	0.75	1.5	1.2	48	0.60	8.5	+	−
100	1.0	2.0	1.6	10	0.57	8.6	−	−
100	1.5	3.0	2.4	3	0.53	8.7	−	+

* Calcium chloride and sodium hydroxide were added simultaneously, dropwise, with stirring.
† Obtained by 5 minutes centrifugation in a clinical centrifuge followed by filtration through filter paper.
‡ + indicates precipitate, hence excess of phosphate ions in supernatant fluid; − indicates no precipitate.

chicken red cell agglutinating (CCA) activity was used as a quantitative measure of influenza virus,[8, 9] but in all cases in which it was also followed by means of nitrogen determinations the results were essentially identical. It can be seen from Table 1 that about 2 cc of 1 M calcium chloride were required to precipitate about 95 per cent. of the CCA activity and that this was accompanied by the precipitation of 0.11 mg of nitrogen per cc of allantoic fluid. With some allantoic fluids as little as 1.5 cc of molar calcium chloride were found sufficient to precipitate 95 per cent. or more of the CCA activity from 100 cc of fluid.

The distribution of CCA activity, total nitrogen and protein nitrogen on treatment of formalized PR8 allantoic fluid with sufficient calcium chloride to precipitate over 95 per cent. of the virus was determined in order to secure information concerning the nature of the precipitate that is obtained. The amounts of reagents and solutions used for the formation and washing of the precipitates are given in Table 2. It can be seen from Table 2 that the washed precipitate contained 0.03 mg of total nitrogen per cc of allantoic fluid and, since this can be presumed to represent protein nitrogen, the precipitates contained about 0.3 mg of protein per cc of allantoic fluid. Infectious allantoic fluid is known[7] to contain about 0.1 mg of influenza virus protein per cc and since most of the CCA activity originally present in the allantoic fluid was present in the precipitate, the protein contained in the precipitate can be presumed to consist of about

TABLE 2

DISTRIBUTION OF NITROGEN AND CCA ACTIVITY ON FRACTIONATION OF FORMALINIZED PR8 ALLANTOIC FLUID BY CALCIUM PHOSPHATE METHOD

Fraction	Experiment 1*				Experiment 2†			
	Total N	Protein N	CCA activity	pH	Total N	Protein N	CCA activity	pH
	mg/cc	mg/cc	units/cc		mg/cc	mg/cc	units/cc	
Formalinized PR8 allantoic fluid ..	0.70	0.16	300	8.1	0.47	0.10	314	8.2
Supernatant liquid of calcium phosphate precipitate ...	0.62	0.11	16	8.0	0.39	0.02	4	8.1
First wash of precipitate.	0.05	0.02	24	7.6	0.05	0.03	18	7.6
Second wash of precipitate	0.01	0.00	2	7.4	0.01	0.01	1	7.6
Suspension of washed precipitate ...	0.03				0.03			

* 250 cc PR8 allantoic fluid, 0.125 cc formalin, 3 cc 1 M sodium phosphate at pH 8, 3.75 cc 1 M calcium chloride and 3 cc 1 M sodium hydroxide were used. Precipitate was washed twice with 125 cc portions of 0.05 M sodium phosphate at pH 7.6. Data are based on volume of allantoic fluid used as starting material.
† 500 cc PR8 allantoic fluid, 0.25 cc formalin, 15 cc 1 M calcium chloride and 12 cc 1 M sodium hydroxide were used. Precipitate was washed twice with 200 cc portions of 0.05 M sodium phosphate at pH 7.6. Data are based on volume of allantoic fluid used as starting material.

[4] J. E. Salk, SCIENCE, 101: 122, 1945.
[5] M. A. Lauffer and W. M. Stanley, *Jour. Exp. Med.*, 80: 531, 1944.
[6] G. L. Miller, M. A. Lauffer and W. M. Stanley, *Jour. Exp. Med.*, 80: 549, 1944.
[7] W. M. Stanley, *Jour. Exp. Med.*, 81: 193, 1945.
[8] G. K. Hirst, SCIENCE, 94: 22, 1941.
[9] G. L. Miller and W. M. Stanley, *Jour. Exp. Med.*, 79: 185, 1944.

one third virus protein and two thirds non-virus protein. It was found by means of total solids determinations on the washed precipitates from allantoic fluid and on precipitates obtained by the reaction between 1 M calcium chloride and 0.1 M phosphate buffer at pH 7 that essentially all the calcium is precipitated as the diphosphate. Therefore the results indicate that when sufficient 1 M calcium chloride is added to 100 cc of allantoic fluid to precipitate about 95 per cent. or more of the virus, the washed precipitate will consist of about 10 mg of virus protein, about 20 mg of non-virus protein and about 200 to 300 mg of calcium phosphate. The virus-calcium phosphate ratio is about 1 to 25 and this can not be decreased because a reduction in the amount of calcium chloride added would result in a loss of virus. The high virus-calcium phosphate ratio operates effectively to prevent concentration of virus to any great extent. Because of this fact and the fact that the precipitates contain a large amount of non-virus protein the method can not be regarded as efficient for either the concentration or purification of influenza virus present in allantoic fluids.

However, because of Salk's[4] finding that calcium phosphate has an adjuvant effect on formalinized influenza virus, a study was made of the precipitation on calcium phosphate of influenza virus[2] and vaccine[7] purified and concentrated by differential centrifugation. In preliminary experiments with active purified PR8 influenza virus over the concentration range of 0.1 mg to 10 mg of virus protein per cc in 0.1 M phosphate buffer at pH 7 it was found that, regardless of the virus concentration, approximately 5 cc of molar calcium chloride were required per 100 cc of virus solution to precipitate 95 per cent. or more of the virus. This finding was confirmed in experiments with a concentrated purified polyvalent influenza virus vaccine prepared commercially and described in an earlier publication.[7] The results obtained with this vaccine (Table 3) demonstrate that when about 95 per cent. or more of the virus is precipitated, the virus-calcium phosphate ratio is about 1 to 70 when virus is precipitated from a solution containing 0.1 mg per cc, about 1 to 7 when virus is precipitated from a solution containing 1 mg per cc and about 1 to 1 when virus is precipitated from a solution containing 10 mg per cc.

It is necessary to carry out the formation of the calcium phosphate precipitate in the presence of the virus in order to secure effective precipitation of the virus for in an experiment in which 1 mg of virus in 1 cc was added to a suspension of precipitate formed by the addition of 0.3 cc of molar calcium chloride and 0.22 cc of molar sodium hydroxide to 9.0 cc of 0.1 M phosphate buffer at pH 7 about 37 per cent. of the virus was found in the supernatant liquid following centrifugation of the mixture in a clinical centrifuge. In another test in which 10 mg of virus in 1 cc were added to a suspension of precipitate formed by the addition of 0.5 cc of molar calcium chloride and 0.38 cc of molar sodium hydroxide to 9.0 cc of 0.1 M phosphate buffer at pH 7 about 28 per cent. of the virus was found in the supernatant liquid. As indicated by the data in Table 3 the corresponding values, when the precipitate was formed in the presence of virus, were 17.6 and 4.8 per cent., respectively. The fact that the precipitate must be formed in the presence of the virus in order to secure effective precipitation of virus and the fact that over a wide range of virus concentration a given amount of calcium phosphate precipitates out essentially the same percentage of virus are of considerable interest.

TABLE 3

PRECIPITATION OF CONCENTRATED, PURIFIED POLYVALENT INFLUENZA VIRUS VACCINE* ON CALCIUM PHOSPHATE

Concentration of virus materials in 10 cc of solution	Volume of reagents added†		Supernatant liquid‡		
				Test with	
	1 M calcium chloride	1 M sodium hydroxide	CCA units per cc	Calcium ions§	Phosphate ions
	cc	cc			
0.1 mg per cc ..			170	+	−
" ..	0.2	0.15	72	+	−
" ..	0.3	0.22	30	+	−
" ..	0.4	0.30	17	+	−
" ..	0.5	0.38	11	+	−
1.0 mg per cc ..			1,700	+	−
" ..	0.4	0.30	174	+	−
" ..	0.5	0.38	82	+	−
" ..	0.6	0.46	38	+	−
10.0 mg per cc ..			17,000	+	−
" ..	0.5	0.38	144	+	−
" ..	1.0	0.77	2	−	+

* Vaccine prepared commercially to contain 10 mg of influenza virus materials per cc in 0.1 M phosphate buffer at pH 7, inactivated with 0.05 per cent. formalin and preserved with 1 : 100,000 phenyl mercuric nitrate. Contains Lee, PR8 and Weiss virus materials in the ratio of 2 : 1 : 1.

† Calcium chloride and sodium hydroxide were added simultaneously, dropwise, with stirring.

‡ Obtained by 5 minutes centrifugation in a clinical centrifuge followed by filtration through filter paper.

§ + indicates precipitate, hence excess of phosphate ions in supernatant fluid; − indicates no precipitate.

Since formalinized influenza virus purified by a single cycle of differential centrifugation can be obtained readily on a large scale[7] at a concentration of about 3 mg per cc it is obvious that such material can be used to prepare virus-calcium phosphate precipitates in which the ratios of virus to calcium phosphate can range from about 1 to 2 to any larger value. If necessary more concentrated virus, which will yield ratios down to 1 to 1, can be obtained. The immunizing potency of different amounts of precipitates containing various virus-calcium phosphate ratios should be determined in order to establish the optimum ratio and the optimum amount of precipitate necessary for the effective immunization of human beings. Since a virus-calcium phosphate ratio less than

about 1 to 25 can not be obtained with allantoic fluid whereas with concentrated virus any ratio down to about 1 to 1 can be obtained, a much more complete study of the adjuvant effect of calcium phosphate on influenza virus vaccines can be made by the use of virus materials concentrated and purified by means of differential centrifugation.

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RECORDING OF SOUNDS PRODUCED BY CERTAIN DISEASE-CARRYING MOSQUITOES

THE destruction of disease-carrying mosquitoes is a problem of the first magnitude. Various methods have been employed such as oiling, draining and the application of larvacides and insecticides. In this connection, the use of D.D.T., both in powder form and as a spray, has been astonishingly effective when used in the field. D.D.T. destroys a variety of insects both harmful and beneficial so that the problem of disturbing the biological balance such as the interference with pollenizing insects, for instance, must be taken into consideration.

When sprayed upon walls of dwellings D.D.T. will repel or kill mosquitoes and other insects for long periods of time. It must not be forgotten, however, that this substance is toxic when ingested. The other methods of mosquito control are often quite expensive and time-consuming.

In order to circumvent these objections, we have approached the problem of mosquito destruction from a somewhat novel angle. It occurred to us that mosquitoes might have characteristic "mating calls." If so, these characteristic sounds, if properly recorded and satisfactorily reproduced, might aid in the attraction of mosquitoes to their death.

We have successfully recorded mosquito sounds which are faintly audible, or completely inaudible to the human ear, and are now able to transmit these sounds, with the end in view of calling specific varieties of mosquitoes to a place of destruction.

The following mosquitoes were employed: *Anopheles quadrimaculatus, Aedes aegypti, Aedes albopictus* and *Culex pipiens*. Colonies of these insects are maintained in the laboratory. The electrical apparatus which is utilized is (1) a microphone, (2) an amplifier of considerable more than usual power, (3) suitable band pass filters and (4) a conventional high quality disk recorder. When the recordings are made, the mosquitoes are placed in a sound-proofed test chamber under conditions of proper temperature and humidity, in order to obtain sensitive recordings under a natural environment.

Certain varieties of mosquitoes such as *Aedes aegypti* have been thought to produce little if any sound. If sounds are produced, they must be outside of the range of human hearing. This we have found to be the case. One may disturb a cage containing 200 or more of this species, yet no audible sounds are heard. One may listen intently in a very quiet room to *Culex pipiens,* but only the very most intense sounds of this species are faintly audible. We have observed that the largest variety of sounds made by this species as well as the others referred to are outside the energy range of normal hearing.

Despite the great variety of sounds, each genus and species have tonal emanations which are so distinctive in character that an experienced observer can not only readily distinguish one genus from another, but with no difficulty at all can also distinguish the males of a species from the females of the same species. Even such closely related species as *Aedes aegypti* and *Aedes albopictus* can be distinguished by sounds alone.

In not a few respects, the sounds of the mosquitoes we have tested are like bird calls. Their variety seems to indicate that they may be in the nature of (a) mating calls, (b) calls warning of danger, (c) calls of anger and other sounds that are similarly functional.

All the sounds that we have recorded to date are in the center of the frequency range of human hearing but far below the energy level required for that purpose. The fundamental tones lie in the range from 250 cycles per second to 1,500 cycles per second. It is interesting to note that all male "voices" so far recorded are much more high pitched than the females. Pitch is a simple means of distinguishing one mosquito sex from another. Female voices contain far more energy than males even when the insects are not in flight.

The most astonishing and important observation of this experiment is that the noise of a single female will cause the males of the same species to burst into an answering chorus. Moreover, when the call of a female is transmitted to two or three males under the circumscribed space of a small test-tube, it has been observed under the microscope that the antennae and hypopygium of the male will turn toward the direction from whence the sound is being transmitted.

Single mosquitoes seemingly do not emanate any sound. Two or more must ordinarily be together before any sounds are generated. This seems to be true regardless of species or of sex. If two mosquitoes of the same sex do not choose to be active, the addition of a mosquito of the opposite sex will often cause activity in the erstwhile silent insects.

In certain mosquitoes, two tones, separated by a