technique six successive generations have been obtained. In the latter case, the infectivity of the blood after six days' culture *in vitro* was demonstrated by inoculation into a monkey. It should be pointed out, however, that in order to obtain subculture with the rocker-perfusion technique, it is necessary to replace about one fourth of the nutrient medium with blood serum. Indeed, we have recently found that better *in vitro* multiplication and growth result during the first 24 hours by the perfusion technique if some serum is present in the nutrient medium. This fact emphasizes one of the basic reasons for our employment of two types of cultivation techniques. We find that the perfusion technique is particularly useful for the study of the nutrient requirements of the parasites, because any low molecular weight material essential for growth and not present in the nutrient fluid will be dialyzed away from the parasite and its deficiency rapidly made apparent. In the rocker-dilution technique, it is obvious that such deficiencies in the nutrient media will not be so readily observable. However, the rocker-dilution technique, because of its simplicity and lack of a Cellophane membrane, has proved very useful in testing the action of antimalarial drugs, immune serum, etc., on the growth of the malaria parasite *in vitro*.

Chemical and metabolic studies have also been made on parasites grown *in vivo* and *in vitro*. Increases have been observed in the content of fatty acids, flavine adenine dinucleotide, total phosphorus, 15-minute hydrolyzable phosphorus, phospholipid phosphorus and nucleic acid phosphorus (by difference) in the red blood cells as their parasite count increases either *in vitro* or *in vivo*. Similar studies on glucose and oxygen consumption and lactate production have also been carried out. We have encountered a striking difference between *in vitro* and *in vivo* grown parasites only in their oxygen consumption. Multiplication of parasites *in vitro* has not been attended by the same increase in oxygen consumption that is observed during multiplication *in vivo*. We have as yet no explanation for this phenomenon, but it may be a reflection of some deficiency or toxic agent in our media or an indication that, if the parasite is well supplied with nutrients, it can exist largely on energy derived from anaerobic processes. In support of the latter explanation is our finding that a gas phase low in O_2 (0.37 per cent. O_2: 5 per cent. CO_2: 94.63 per cent. N_2) permits at least as good growth and multiplication *in vitro* as in 95 per cent. air: 5 per cent. CO_2. Definitely detrimental to *in vitro* growth is a gas phase high in oxygen content (95 per cent. O_2: 5 per cent. CO_2).

The composition of the nutrient medium employed and given in Table 1 was arrived at by *a priori* reasoning. We can not say at present how many of the components of this medium are essential for growth of the parasite. Omission of the proteose peptone is, however, definitely detrimental to growth and multiplication. Recent experiments indicate that para-amino benzoic acid is probably the chief essential growth component furnished by the proteose peptone, a fact which may help to explain the observation of Coggeshall[5] that sulfanilamide will eradicate *P. knowlesi* infections in monkeys.

The techniques described here are also now being applied with the assistance of Dr. J. W. Ferrebee to the *in vitro* cultivation of human malaria parasites. Results to date have been encouraging and will be reported at a later time.

ERIC G. BALL
CHRISTIAN B. ANFINSEN
QUENTIN M. GEIMAN
RALPH W. MCKEE
RICHARD A. ORMSBEE

THE ANTIBODY RESPONSE OF SWINE TO VACCINATION WITH INACTIVATED SWINE INFLUENZA VIRUS[1]

THE two human types of influenza virus, A and B, and the swine type of the agent have been obtained[2, 3, 4] in preparations of high purity by ultracentrifugation of the chorio-allantoic fluid of virus-infected chick embryos. A high degree of concentration and partial purification of the agents can be effected by sedimentation in the Sharples centrifuge,[5, 6] and procedures have been devised for practical large-scale production of virus for preparation of vaccines.[6, 7]

[5] L. T. Coggeshall, *Jour. Exp. Med.*, 71: 13, 1940.

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[2] A. R. Taylor, D. G. Sharp, D. Beard, J. W. Beard (Consultant to Secretary of War and Member, Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army), J. H. Dingle and A. E. Feller, *Jour. Immunol.*, 47: 261, 1943.

[3] D. G. Sharp, A. R. Taylor, I. W. McLean, Jr., (Member, Commission on Epidemiological Survey, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army), D. Beard, J. W. Beard, A. E. Feller and J. H. Dingle, *Jour. Immunol.*, 48: 129, 1944.

[4] A. R. Taylor, D. G. Sharp, I. W. McLean, Jr., D. Beard, J. W. Beard, J. H. Dingle and A. E. Feller, *Jour. Immunol.*, 48: 361, 1944.

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

[6] A. R. Taylor, D. G. Sharp, I. W. McLean, Jr., D. Beard and J. W. Beard, *Jour. Immunol.*, in press.

With access to purified, concentrated influenza virus, quantitative studies have been undertaken on the antibody-inducing capacity of influenza vaccines using swine as the test animal and the swine influenza virus as the antigen. Swine are a natural host[8] for the swine influenza virus, which is closely similar morphologically[9] and physio-chemically[10,11] to the human types of influenza virus. In contrast with conditions in man,[12] swine can be procured without previous experience with the influenza virus, and experiments with these animals under controlled conditions should give a good estimate of the effects of the vaccine itself.

The first study was made to determine the relation of virus mass (as vaccine) to host antibody response measured by the capacity of the serum taken at weekly intervals to inhibit the hemagglutinin reaction.[13] Two types of vaccines,[14] namely, formalin-inactivated and ultraviolet light-inactivated virus, were given subcutaneously in 1 ml volumes in doses of 0.125, 0.25, 0.5, 1.0 and 2.0 mg of virus per ml. Each dose was given to 12 pigs. It was found that within the range of dosage used, 0.1 to 5.0 mg per 100 lb of animal weight, a 10-fold increase in dose of the formalin vaccine produced only a 2.5-fold increase in titer, and a similar increase in the dose of ultraviolet light vaccine resulted in a 1.6-fold increase in titer. The titer reached a peak within 7 to 9 days and then declined within 3 weeks to a level about three 2-fold dilution intervals below the maximum average titer attained. Doses greater than 0.5 mg appeared to cause general reactions in the animals manifested chiefly by loss of weight.

These results showed clearly that though antibody titer was related to dose, the response was by no means directly proportional to dose, and further the response was short-lived with any dose given. For this reason a second vaccination was made with the same doses about 6½ weeks after first vaccination. The results were qualitatively similar to those of the first vaccination, and a 10-fold increase in dose of either vaccine was associated with a 1.6-fold increase in titer. In marked contrast with the results of a single vaccination, however, much higher titers were obtained, and the decline in titer subsequent to second vaccination was much slower. Higher and more lasting titers were obtained with 0.25 mg of vaccine divided into two 0.125 mg doses, for example, than by an amount almost ten times as great given in a single injection. No significant difference was found between the effects of the vaccine prepared by the two methods of inactivation.

Further investigation was made of repeated vaccination particularly with respect to the optimum interval between the injections. For this, 64 pigs were given 0.5 mg of formalized virus, and at intervals of a week successive groups of 16 animals were revaccinated. The results showed that both the titer attained and the amount of antibody produced were greater the longer the interval between vaccinations. Furthermore, the greater the interval, the greater were the amounts of circulating antibody retained through the 6 weeks follow-up period. Repetition of vaccination after only 1 week produced little increase in titer over that following first vaccination. In fact, 7 of the 16 pigs revaccinated at the one week interval showed no increase, or an actual decrease in titer.

Experiments were made to test the possibility that higher titers might be obtained and maintained by vaccinating with vaccine adsorbed on alum. The effects of a single injection of 0.5 mg of formalized virus adsorbed on different amounts of alum, from 0.3 to 1.2 per cent. were similar in magnitude to those of a single injection of vaccine without alum. A striking difference was the delay of peak response until the second week post-vaccination; following this, antibody was lost at nearly the usual rate. The height of the peak response increased as the concentration of alum decreased, but with the highest concentration of alum tested a somewhat higher and more uniform titer was maintained throughout the 7 weeks follow-up period. All the animals showed areas of local induration at the site of inoculation that gradually subsided, but could still be detected in nearly all instances at the end of seven weeks.

Vaccinations repeated at two and four weeks with 0.5 mg of virus adsorbed on 1.2 per cent. alum gave results similar in magnitude to those obtained with vaccine containing no alum. Here, also, two vaccinations, four weeks apart, gave the highest titers, and the decline in titer was not much slower than that in animals receiving vaccine without alum. It appears doubtful that the use of alum influenced significantly either the antigenic capacity of formalinized swine influenza virus or the maintenance of antibody titer.

The findings reveal an efficiency of repeated small doses of vaccine in swine far greater and longer-lived than that of a single large dose, and a considerable dependence of the degree of effect on the length of the interval between vaccinations. Formalin and ultraviolet vaccines behaved alike, and adsorption on

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[13] G. K. Hirst, *Jour. Exp. Med.*, 75: 49, 1942.
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alum did not greately enhance the titer or prolong the antibody level.

I. W. McLEAN, JR.,
1st Lieut., M.C., A.U.S.
DOROTHY BEARD
A. R. TAYLOR
D. G. SHARP
J. W. BEARD

DEPARTMENT OF SURGERY,
DUKE UNIVERSITY SCHOOL OF MEDICINE

ROOTING GREENWOOD CUTTINGS WITHOUT SUNLIGHT UNDER FLUORESCENT LAMPS

OVER a century ago, improvement in heating devices led to the change from opaque structures with some glass-covered openings to the greenhouse of the present. The improved illumination thereby obtained seemed to compensate for the increased fuel requirement and more frequent watering and more constant care this change entailed. For many years, though fuel and labor costs have increased markedly and new types of illumination have been developed, there has been no essential change in the type of house used for growing plants, and propagation of plants has been carried on in the same houses, or slight modifications of them, as are used for growing. High fuel costs in winter and excessive insolation in summer have been accepted unquestionably.

Recent studies made at the U. S. Plant Introduction Garden of the Division of Plant Exploration and Introduction, U. S. Department of Agriculture, Glenn Dale, Maryland, indicate the desirability of investigation of the possibility of using opaque structures for the rooting of cuttings. Such structures if partly or wholly underground could be heated economically in winter and also could avoid the high temperatures which are often a source of difficulty in the summer.

Some experiments conducted during the summer of 1942 indicated that more rapid rooting of cuttings of *Weigela floribunda* (Sieb. and Zucc.) C. A. Mey, *Ligustrum ovalifolium* Hassk. and *Chrysanthemum morifolium* Ram. could be obtained in a basement darkroom under continuous illumination from a 100 watt mazda lamp than in conventional propagation equipment in a greenhouse. In a continuation of these experiments using more efficient lighting equipment, particularly good results were obtained with small detached propagation cases of opaque material, each fitted with a single 30 watt fluorescent lamp. These cases were approximately six feet in length, two feet wide and three feet high, and the lamp was placed 10 inches above the rooting medium, which in this instance was a coarse grade of vermiculite. Automatic control of the bottom heat in the rooting medium was provided by means of a lead-sheathed soil-heating cable controlled by a thermostat. The small amount of heat given off by the lamp was absorbed largely by the air space above it and did not raise the temperature of the air around the cuttings. In other experimental frames using lamps of higher wattage, the cuttings were protected from heat by means of a glass partition between the cuttings and the lamp. Both the white and the daylight quality lamps have been used successfully. The operating cost was low because of the high efficiency of the fluorescent lamps.

Uniform relative humidity (approx. 80 per cent.) was maintained easily within the cases because of the constant light and temperature (75° F.). In the conventional greenhouse, on the other hand, the constant fluctuations cause serious difficulty in maintaining a uniform humidity.

The results obtained in rooting cuttings in these frames have been exceptionally good, and have surpassed those in similar propagating frames within an ordinary greenhouse, when the rooting medium and the temperatures have been the same in both locations. The cuttings tried include those of various species of *Citrus, Cinchona, Severinia, Hibiscus, Bougainvillea* and unrooted divisions of *Cymbopogon citratus* DC.

In comparing rooting under 16-hour daily photoperiods with that obtained under continuous illumination, the responses were often superior in one or the other day-length according to the individual species. The difference was negligible with cuttings of some plants. These responses can be changed by treatments with growth substances in some manner not explicable at present. For instance, cuttings of the Chinotto orange, *Citrus Aurantium* Linn. var. *myrtifolia* Ker-Gawl., following a treatment by dipping the bases in a 50 per cent. solution of ethyl alcohol containing five mg of potassium indole butyrate per ml rooted heavily under a 16-hour photoperiod in only two weeks, but rooting was slight and greatly delayed under continuous illumination. Moderate and approximately equal rooting of untreated cuttings was obtained in both day-lengths. On the other hand, the identical treatment of growth substance on cuttings of rough lemon, *Citrus Limon* Linn., produced a heavy rooting response under continuous illumination, but rooting was inhibited under the 16-hour photoperiod. As with the Chinotto orange, the rooting of the untreated cuttings was practically equal under both periods of illumination.

V. T. STOUTEMYER
ALBERT W. CLOSE

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING

F. L. O'ROURKE

SOIL CONSERVATION SERVICE,
U. S. DEPARTMENT OF AGRICULTURE