Red or Alpha Naphtholphthalein for the alkaline. Accordingly, these indicators were used in preparing the calibration curve.

The calibration curve was constructed by mixing CO_2 with air to give an atmosphere of known pCO_2; the volume of each component was measured in a standardized flow meter. The CO_2 content was checked by absorption of the CO_2 in a measured volume of the atmosphere in 0.1 N NaOH contained in a Truog tower and titrating the excess alkali to the Thymol Blue end-point. At the same time, the atmosphere was bubbled through about 4 cc of standard $NaHCO_3$ solution plus indicator in a 13 mm Board of Health test-tube fitted with an inlet tube drawn to one mm capillary tip. The equilibration of the $NaHCO_3$ solution required about 5 minutes, after which the test-tube was transferred to the comparator and the pH estimated. Duplicate determinations, which never varied more than 0.05 pH units, were made with each indicator used.

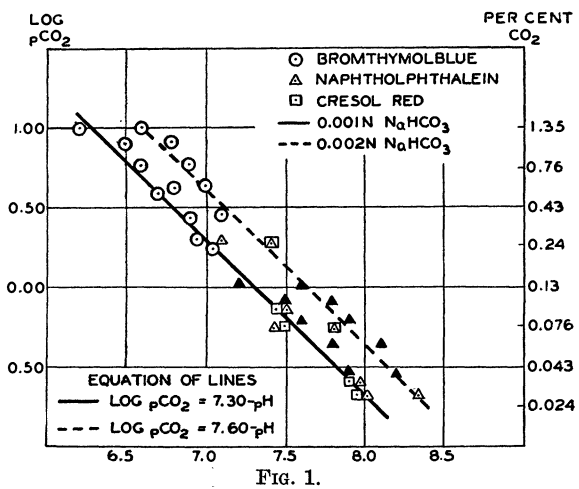

FIG. 1.

The calibration curve is given in Fig. 1; two concentrations of $NaHCO_3$ were used to give a check on the systematic error of the indicators. The solid symbols represent values obtained with 0.0107 N and 0.0214 N $NaHCO_3$; the pCO_2 corresponding to each of these points was divided by 10 before plotting. The curve, as given, is for use with 0.001 N or 0.002 N $NaHCO_3$, but by use of 0.0107 N or 0.0214 N $NaHCO_3$ solution, pressures of CO_2 ten times those indicated on the ordinates can be estimated.[2] The values for the percentage of CO_2 were calculated on the assumption of an average barometric pressure of 740 mm. It is to be noticed that the points lie along a line with the proper slope of unity and are displaced from each other by the theoretical value, *viz.*, 0.3 pH units.

Use of the method in routine analysis of air in a greenhouse is illustrated by the following example: To 20 cc of standard $NaHCO_3$ solution is added 1 cc of indicator solution (Brom-thymol-blue for a pCO_2 greater than 0.15 per cent.) and approximately 4 to 5 cc of the mixture placed in a 13 mm test-tube provided with a drawn-out inlet tube. The outlet tube is attached to a bottle containing water and fitted with a syphon. About 500 cc of air is drawn through the $NaHCO_3$ solution by displacement of water in the syphon, the pH determined by comparison with the proper color disk, then the pCO_2 read from Fig. 1. For routine analysis, a table in 0.05 pH units can easily be constructed for average barometric pressures by means of the equations of the lines given in Fig. 1. If the pH is read to 0.05 units, the error in the determination will be 10 per cent. or less.

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SPECIAL ARTICLES

A VIRUS ENCOUNTERED IN THE STUDY OF MATERIAL FROM CASES OF ENCEPHALITIS IN THE ST. LOUIS AND KANSAS CITY EPIDEMICS OF 1933

A VIRUS has been disclosed by the intracerebral inoculation of special mice with brain tissue from fatal cases of encephalitis occurring in St. Louis and Kansas City during August and September, 1933.[1] The mice employed, bred in our laboratory as part of a study of inherited factors in susceptibility and resistance to infectious diseases,[4] had previously proved susceptible to an infectious encephalitis of sheep.[5] It seemed possible, therefore, that experiments with these animals might throw light on the nature of the human encephalitis.

Brain tissue preserved in 50 per cent. glycerol from each of eight St. Louis cases[6] was ground in a sterile mortar, diluted in 0.85 per cent. salt solution to approximately a 10 per cent. solution by weight, cultured aerobically and anaerobically to detect the pres-

[1] Muckenfuss, Armstrong and McCordock have already reported (foot-notes 2 and 3) that a condition similar to the St. Louis encephalitis has been reproduced in monkeys and has been maintained through five passages.

[2] J. P. Leake, *J. A. M. A.*, 101: 928, 1933.

[3] R. S. Muckenfuss, C. Armstrong and H. A. McCordock, *Public Health Rep.*, 48: 1341, 1933.

[4] L. T. Webster, *J. Exp. Med.*, 57: 793, 1933.

[5] L. T. Webster and G. L. Fite, *Proc. Soc. Exp. Biol. and Med.*, 30: 656, 1933.

[6] Dr. Muckenfuss collected and sent material to us in New York. This assistance is gratefully acknowledged.

ence of bacteria, and then injected intracerebrally in 0.03 cc quantities into two white-face and two Swiss mice.

The mice remained well and active for four days. Two mice from Case 3 were dead on the sixth day, and one in convulsions was killed. One mouse from Case 4 with convulsions on the sixth day and one prostrate on the ninth day were killed. One mouse from Case 5, prostrate on the eighth day, was killed; and one from Case 8 was dead on the ninth day. All these, with one exception, were white-face mice. The others, including all mice from Cases 1, 2, 6 and 7, remained well.

The brains from the dead mice were removed aseptically, cultured aerobically and anaerobically and found sterile, treated as before, and injected intracerebrally into two white-face and two Swiss mice. In each instance, the injected mice remained well three days and were all dead by the fifth day.

The four strains of virus have given uniform results in white-face, Swiss and other susceptible strains of mice[4] for as many as fifteen passages. After a three- or four-day healthy period, all animals show ruffled fur and hyperesthesia. Tremors, convulsions and prostration develop rapidly and progress to a fatal conclusion in from five to nine days.

The lesions in the central nervous system consist for the most part of accumulations of mononuclear cells in the pia, about the blood vessels of brain and spinal cord and in scattered foci. The pyramidal cells of the cornu Ammonis and lobus piriformis are injured. Some of the cells are ameboid in outline; others are necrotic, thus disturbing the regular architecture of these regions. Certain nerve cells of the basal ganglia and anterior horns of the spinal cord appear damaged and collared by mononuclear cells.

When the mouse brain virus is instilled intranasally in 0.03 cc quantities, fatal signs develop in practically 100 per cent. of white-face and Swiss mice. After a five- to six-day incubation period, the tremors and convulsions ensue, terminating in death by the tenth day. The lesions of the central nervous system are similar to but less intense than those in intracerebrally injected mice.

Intracerebral injections of the mouse brain virus into Rockefeller Institute stock mice and specially bred virus-resistant mice produce disease in a relatively small percentage of animals.

Macacus rhesus monkeys, given 1 cc of mouse brain virus intracerebrally, have invariably shown significant elevations of temperature in from seven to nine days, lasting three to five days and followed by either hyperirritability or apathy. In some cases, coarse tremors are present. Hyperirritable animals later become weak and drowsy; apathetic animals continue so for at least fourteen days. This condition has been induced regularly for four monkey passages. Blood and spinal fluid drawn from four monkeys during the febrile period and injected intracerebrally into mice has had no effect; brain tissue from monkeys etherized seven to twenty-eight days after injection, when so treated, has invariably brought about the characteristic fatal disease in mice.[7] The central nervous system lesions in the injected monkeys consist of collections of mononuclear cells in the pia and perivascular collaring of mononuclear cells and foci of mononuclear cells and injured nerve cells scattered throughout the brain and cord, together with injury to some of the nerve cells of the basal ganglia and anterior horns.

The different strains of virus produce identical effects in mice. They are active in dilutions as high as 10^{-5} injected intracerebrally. Berkefeld N candle filtrates in dilutions as high as 1/2000 likewise prove fatal.

Brain tissue preserved in glycerol from two fatal cases of encephalitis in Kansas City in September, 1933,[8] was injected intracerebrally into white-face mice. One mouse from one case in convulsions on the ninth day was sacrificed and its brain prepared and passed intracerebrally to two white-face and two Swiss mice. Six days later all were having convulsions or were prostrate. This virus has proved identical with the St. Louis strains.

The following statement may be made on the nature of these viruses. The possibility of their being a native mouse virus is unlikely because mice in the breeding room have shown no such disease, because sections from at least sixty normal white-face mice have not disclosed lesions of the central nervous system, because brain to brain passages in these animals for the purpose of bringing to light a similar agent from cases of common cold, acute rheumatic fever and poliomyelitis have been consistently negative, and finally, because repeated attempts at isolating the present strains of virus from the original human tissues gave positive results in the cases which previously were positive, and negative results in the cases which previously were negative, and another later attempt, when the active human brain tissue was at least six weeks in glycerol, gave negative results in all cases. The agent does not appear to be related to louping-ill virus of sheep because sera protecting mice against at least 100 lethal doses of this virus have no effect upon the present one. Monkeys injected with it do not show the cerebellar signs of incoordination and lesions so characteristic of louping-ill.

[7] Muckenfuss, Armstrong and McCordock report (footnote 3) that the disease in monkeys with which they are working may be transferred and established in mice.

[8] Dr. Paul Stookey sent us the brain tissue and convalescent sera from Kansas City cases. We gratefully acknowledge this assistance.

The virus does not appear to be related to vesicular stomatitis or to equine encephalomyelitis, since it is non-pathogenic for guinea pigs when injected intracerebrally and because it is not neutralized when injected in mice with immune sera for equine encephalomyelitis and vesicular stomatitis.[9] The virus appears unrelated to herpes virus since rabbits show no effects following intracerebral, corneal and intradermal injections.

Sera from convalescent St. Louis[10] and Kansas City cases, in contradistinction to sera from non-contact healthy adults, appear to possess specific protective substances. Virus suspensions were mixed with undiluted human sera to give final virus dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The mixtures were incubated at 37° for two hours and inoculated intracerebrally into four Swiss mice. Six sera, including one positive and one negative control, were tested at one time. The table summarizes a protocol of one of these titrations.

Thus far, sera from eight normal adults in New York tested twice have shown no protective qualities. Sera from eight St. Louis encephalitis convalescent cases, tested twice, however, all show definite protective properties.

PROTECTION TEST

Sera	Virus-Serum Dilution 10^{-3}	10^{-4}	10^{-5}	10^{-6}
J., Normal New York	5*, 5, 6, 8	7, 7, 8	8	
K., Normal New York	4, 5, 5, 8	5, 5, 5, 6	7, 7, 7	
R., Normal New York	5, 5, 5, 7	5, 5, 5, 7		
No. 10 St. Louis Convalescent	6			
No. 9, St. Louis Convalescent	7, 9, 10			
No. 33, St. Louis Convalescent	8, 10	8		

* Duration of life of mouse in days. Blanks indicate mice remained healthy.

The facts presented would indicate that the active agent is a specific filtrable virus, etiologically related to the encephalitis prevailing in St. Louis and Kansas City and that one possible mode of its transmission is by way of the upper respiratory tract. Final decision on these questions must be reserved, however, until the work in progress has been completed.

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[9] The writers are indebted to Dr. H. R. Cox for the immune sera used and assistance in these tests.

[10] These sera were obtained from cases at the St. Louis City Isolation Hospital by Dr. S. Weisman through the courtesy of Dr. J. Eschenbrenner.

THE EXISTENCE OF NON-CHROMOSOMAL INFLUENCE IN THE INCIDENCE OF MAMMARY TUMORS IN MICE[1]

THE object of this communication is to record the existence of extra-chromosomal influence, extending for more than one generation and affecting the natural incidence of spontaneous mammary tumors in mice.

The data are based on four independently conducted experiments which have continued over a period slightly in excess of three years. Seven distinct inbred strains of mice (six derived from *Mus musculus,* and one the direct descendants of wild *Mus bactrianus*) have been used. The results in all four experiments are consistent with, and confirmatory of, one another. Since in our experience females only have formed spontaneous mammary tumors, that sex alone is included in the tabulation of tumor incidence. More detailed papers on the different experiments will later be published. The present note seeks merely to record certain facts of general interest and application.

The opportunity to detect extra-chromosomal influence is offered by a comparison of the incidence of spontaneous mammary tumors among the female mice derived from reciprocal crosses between distinct "high tumor" and "low" or "non-tumor" strains. Although no mammary tumors have been recorded in certain strains designated as "low tumor," it seems better to use that term as an admission that the possibility of the future appearance of such neoplasms is recognized.

Since in such reciprocal crosses the chromosomal constitution of F_1 females is similar (both as regards sex chromosomes and autosomes) it follows that any significant difference in tumor incidence which may exist between the two types of cross is extra-chromosomal. If this difference continues beyond the F_1 generation, direct transmission through the extra-chromosomal portion of the germ-cell is clearly indicated.

In the following table the incidence of spontaneous mammary tumors in F_1 females is recorded in four experiments (A, B, C, D). The reciprocal crosses are shown in each case. In every instance the incidence of spontaneous mammary tumors in F_1 is strikingly and significantly higher when the cross is made between a female from a high tumor strain and a male from a low tumor strain, than it is when the cross is made in the reciprocal manner (low tumor line ♀ × high tumor line ♂). In experiments A and

[1] Acknowledgment of a grant from the American Academy of Arts and Sciences in partial support of this work is hereby made.