

produced a reddened induration, one cm. in diameter, which was followed by desquamation. The strain which was nontoxic for paramecia produced no effect in the skin of the guinea pig. This experiment indicated that the toxic and non-toxic strains acted similarly on paramecia and guinea pigs (Table III).

TABLE III. *Action of Diphtheria Toxin and Antitoxin on Paramecia.*

Paramecium Suspension Parts	Parts	Parts	Paramecia Living After		
			3 Hrs.	5 Hrs.	24 Hrs.
5 Culture Medium,	4 Wheat Water,	1	+++	+++	+++
5 Diphtheria Toxin,	4 " "	1	++	0	0
5 " "	4 Normal Horse Serum	1:200, 1	++	++	0
5 " "	" " "	1:400, 1	++	0	0
5 " "	" " "	1:800, 1	++	0	0
5 " "	4 Diphtheria Antitoxin ¹	1:200, 1	+++	+++	+
5 " "	" "	1:400, 1	+++	+++	+++
5 " "	" "	1:800, 1	+++	+++	+++
5 " "	" "	1:1600, 1	+++	+++	+++
5 " "	" "	1:3200, 1	+++	+++	++

+++ = All paramecia alive.
 ++ = About half of paramecia alive.
 + = Almost all paramecia dead.
 0 = All paramecia dead.

¹ Each cc. contained 2000 units of antitoxin. The lowest dilution of antitoxin in the mixtures contained approximately 0.29 units of antitoxin and the highest dilution contained approximately 0.0127 unit.

Summary: These experiments show that paramecia can be used to determine toxin production of certain bacteria (diphtheria bacillus, measles diplococcus, scarlet fever streptococcus) in a crude way only because the toxins are not sufficiently potent to be diluted, but that paramecia may be helpful in determining the strength of anti-toxins.

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Experiments in Filtration of the Virus of Avian Molluscum.*

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There are conflicting statements in the literature regarding the filterability of the virus of *Avian molluscum*. Some observers reported the presence of the virus in Berkfeld but not in porcelain

* These experiments were done in part at the Lister Institute, London, under the direction of Dr. J. C. G. Ledingham, whose advice is gratefully acknowledged.

filtrates, while some have obtained the opposite results. Others have found the virus only infrequently in filtration experiments. Findlay,¹ the latest observer, found that the virus could be passed easily through Berkfeld V filters. He used a virus of much higher titer (1:10,000) than the one employed in the experiments here reported and also used young chicks (less than a week old) as his test animals, as these were found to be more uniformly susceptible than adult hens. Mudd² described a method for controlling filtration technic and an arrangement of apparatus which would aid in making definite observations of the ability of a virus to pass through filters. This method was used in the present series of experiments with the virus of *Avian molluscum*.

Two filters, each unused except for preliminary pressure tests of Berkfelds V, N and W, Mandler, Seitz and plaster filters with neutral charge were used. A virus suspension in normal salt solution, centrifuged until it was almost clear, was passed through these filters. The first cc. of the filtrate was kept for animal tests, the next 10 cc. portion was discarded and the 12th cc. portion kept. A detailed record was kept of the time of filtration, maximum and minimum pressures for each portion of the filtrate, and sterility tests made of the 3 portions of each filtrate. Intracutaneous tests with 0.1 cc. of each filtrate, in duplicate, were made on the combs of adult hens, giving 4 inoculations of each portion of the filtrate for each type of filter. The unfiltered virus had a titer of 1:100 when given with this method.

In the Berkfeld W filtrations, the pressures varied from 100 to 360 mm. Hg, and the time for the 1 cc. portions to pass through 2 to 7 minutes. Virus could not be demonstrated in these filtrates. In the Berkfeld N filtrations, the pressures varied from 50 to 190 mm. Hg, and the time of filtration 2 to 3 minutes. Virus could not be demonstrated in these filtrates. In the Berkfeld V filtrations, the pressures varied from 90 to 240 mm. Hg, and the time necessary to obtain the filtrates from 1 to 5 minutes. No lesions were produced with the inoculations of these filtrates. In the Seitz filtrations, the pressures varied from 80 to 380 mm. Hg, and time of filtration from 3 to 7 minutes. In one case only the 12th cc. contained virus but this filtrate proved to be contaminated. A number of filtrations were done with the plaster filters having a neutral charge. In no case did the pressure exceed 110 mm. Hg, and the time of filtration more than 3 minutes, but all filtrates except the 1st cc. portion in one

¹ Findlay, G. M., *Proc. Royal Soc.*, 1928, cii, 354.

² Mudd, Stuart, *Proc. Soc. Exp. Biol. and Med.*, 1927, xxv, 60.

case proved to be contaminated; nevertheless the virus could not be demonstrated in any of the filtrates.

A number of the Berkfeld filtrates were inoculated again into the combs of hens by the scratch method and no lesions appeared. Since it was possible that the virus might have been present in the filtrates in insufficient amounts to produce macroscopic lesions, hens which had been inoculated with the filtrates and had given negative results were re-inoculated with unfiltered virus and in each case typical lesions ensued showing that there was, at least, insufficient virus in the filtrates to immunize the animals.

Of several filtrations with Chamberland L1 (bis) filters, using a less diluted and more turbid suspension of the virus which had a titer of 1:1000, it was possible to obtain the virus in an uncontaminated filtrate in one instance. In this case 10 cc. of a 1:20 dilution of horse serum was passed through the filter preliminary to the virus filtration in an effort to satisfy the absorptive power of the filter as recommended by Stockman and Minette.³ This filtrate produced lesions at a dilution of 1:100 by the scratch method. The pressure necessary for the filtration varied between 600 and 650 mm. Hg, and 90 minutes were taken to obtain 2 cc. of the filtrate. The results of this filtration agree with those of Ledingham,⁴ who found that this virus would pass the same type of filter.

Nicolle and Adil Bey⁵ found the virus of vaccinia to be filterable after the virus had been subjected to the action of trypsin. Findlay¹ was not able to confirm this. This method was tried in one experiment with *Avian molluscum* virus. A potent trypsin was added to 1:100 dilution of the virus and incubated at 38° for 3 hours before filtering through a Berkfeld V candle. Duplicate injections of this filtrate in a hen produced no lesions.

Levaditi and Nicolau⁶ reported the successful passage of a number of viruses, including vaccinia, through collodion membranes which retained certain toxins and enzymes but passed certain antibodies. Since they did not use the virus of *Avian molluscum*, 25 filtrations of this method were tried. Membranes of nitro-cellulose were cut in discs to fit a Seitz filter. The period of evaporation and of holding the discs in water before use, varied, as did the pressure used in the filtration process. As the virus suspension contained a certain amount of hen's blood the filtrate was tested in each case against a hen's blood precipitin produced in a rabbit, for permea-

³ Stockman, S., and Minett, F. C., *J. Comp. Path. and Therap.*, 1926, xxxiv, 1.

⁴ Ledingham, J. C. G., *J. State Med.*, 1925, xxxiv, 125.

⁵ Nicolle, M., and Adil Bey, *Compt. Rend. Acad. Sci.*, 1906, cxliii, 1196.

⁶ Levaditi, C., and Nicolau, S., *Compt. Rend. Acad. Sci.*, 1923, clxxvi, 717.

bility of the membrane. Due to the necessary handling of these membranes, sterile filtrates were obtained in 12 instances only. The results on animal inoculations showed the virus to be present in 2 filtrates but in each of these the filtrate was not sterile and the membrane failed to retain the precipitinogen. Here again, re-inoculation with an unfiltered virus in a hen which had received 10 filtrates with negative results, resulted in typical lesions.

Summary: By following the method of Mudd it was found that the virus of *Avian molluscum* was not obtained in filtrates through Berkfeld V, N and W types, Mandler, Seitz and neutral plaster filters. In one instance it was obtained in Chamberland L1 (bis) filtrates. Adult hens were used in each case as test animals. After tryptic digestion the virus was not found in a filtrate from a Berkfeld V filter. This was also true with collodion membranes which would retain a precipitinogen.

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Isolation of Insulin in Crystalline Form from Fish Islets (Cod and Pollock) and from Pig's Pancreas.*

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We have succeeded in obtaining insulin in crystalline form with relative ease from the islets of fishes. These crystals are practically identical in physiological activity and in sulphur content with the crystals prepared from beef insulin.

The crystallization of insulin from pig's pancreas is more difficult. About 2 years ago Professor Abel encountered difficulties in crystallizing insulin from several of the Lilly preparations. He subsequently learned that these extracts had been made from the pancreatic tissue of both pigs and beeves. Following this observation extracts made only from the pancreas of beeves were used until the present opportunity of working with extracts made from the islet tissue of fishes¹ and from the pancreas of the pig,² pre-

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¹ The islet material was kindly collected for us by the General Foods Company, Gloucester, Mass.

² The material for this work was kindly supplied to us by the Lilly Research Laboratories, Indianapolis, Ind.