hypophysis of iodine deficient animals was suggested by histological examination of their hypophysis. These glands showed a decreased percentage of acidophils with an increased number of basophils, as is the case with thyroidectomized animals.[17, 18] Since this hypophyseal picture in thyroidectomized animals is indicative of excessive release of thyrotropic hormone,[16, 19, 20] it is likely that it bears the same significance in animals lacking iodine. Goitrous thyroids would thus result from hypophyseal modifications. However, the possibility of a direct action of iodine on the thyroid is not excluded.[21]

Conclusion. The hyperplastic thyroids of rats given an iodine-deficient diet acquire an increased ability to fix iodine. The thyroid and hypophyseal changes in these goitrous animals indicate an excessive release of thyrotropic factor from their hypophysis.

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Use of Hamster (*Cricetus auratus*) for Detection of Influenza Virus in Throat Washings.*

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One of us has reported[1] that the hamster *(Cricetus auratus)* gave a specific immune response following intranasal inoculation of throat-washings taken from persons acutely ill with influenza A. These observations have now been extended, not only with regard to the immune response of the hamster but also as to its use in adapting influenza A virus to mice.

Experimental Material. The throat-washings used in these experiments were collected from persons presumably ill from influenza during the epidemic which occurred in the Argentine in July, 1940. The throat-washings were taken with 20 cc of an equal mixture of

17 Marine, D., Rosen, S. H., and Sparke, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 803.

18 Sharpless, G. R., and Hopson, E. M., *Endocrinol.*, 1940, **27**, 129.

19 Loeser, A., *Arch. Exp. Path. u. Pharm.*, 1934, **176**, 697.

20 Starr, P., Rawson, R. W., Smalley, R. E., Doty, E., and Patton, H., *West. J. Surg.*, 1939, **47**, 65.

21 Chapman, A., *Endocrinol.*, 1941, **29**, 680.

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1 Taylor, R. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 541.

0.85% NaCl and buffered broth (pH 7.4). They had been preserved for a period of 6 to 8 months at a temperature of approximately −75°C in a thermos or cold cabinet[2] containing CO_2 ice.

For the purpose of serological diagnosis, acute and convalescent blood samples were taken from each person from whom a throat-washing was secured.

Method. Inoculation. The hamsters were inoculated intranasally with 0.4 cc of throat-washings while under light ether-anesthesia. As it had been shown that the intranasal instillation of a bland fluid would increase the quantity of virus in the lungs of mice which had previously received a sublethal dose of influenza A virus,[3] the hamsters were again placed under light ether-anesthesia 2 to 3 days after the inoculation of the throat-washings, and 0.4 cc of 0.85% NaCl solution was instilled into the nose.

The ferrets used for comparison were similarly inoculated with 1 cc of the throat-washings. No second instillation was given to these animals.

From animals used for testing the immune response, a blood sample was taken by cardiac puncture preceding and 2 weeks following inoculation.

The *neutralization-test* was employed for determining the immune response by inoculating mice with varying dilutions of the serum mixed with a standard quantity of virus. It was found advisable to use a relatively small dose of virus, not exceeding 100 MLD to detect consistently the increase of neutralizing antibodies in the sera of the hamsters.

Mouse-passage. For the adaptation of the virus to mice, the hamsters were sacrificed by chloroform-anesthesia on the fourth day following inoculation of the throat-washings, and the turbinates and lungs were removed with aseptic precautions. The turbinates were ground in a porcelain mortar with a small amount of alundum and suspended in 0.75 cc of buffered broth (pH 7.5). The lungs were likewise emulsified and suspended in 4 volumes by weight of broth. Following centrifugation, equal quantities of the supernate of the turbinate- and lung-suspensions were mixed and 0.05 cc was inoculated intranasally into each of 4 mice while they were under ether-anesthesia. On the second or third day 0.05 cc of 0.85 NaCl solution was similarly administered intranasally. On the fourth day the mice were sacrificed. Their lungs were emulsified as above described, and with this suspension 4 additional mice were inoculated,

[2] Horsfall, F. L., Jr., *J. Bact.*, 1940, **40**, 559.

[3] Taylor, R. M., *J. Exp. Med.*, 1941, **73**, 43.

and the passages continued until 5 passages had been completed. If pulmonary lesions simulating viral infection were observed, further passages were made until the virus was sufficiently virulent for titration against a known immune serum which constituted the final criterion for identification of the virus.

Results. Twelve throat-washings were administered to ferrets and hamsters to determine the immune response in these animals. Ten were from persons who according to serological diagnosis had influenza A. Nine of these 10 produced a positive reaction to influenza A virus in the ferret and hamster, while one was negative to both species of animals. One throat-washing was from a person with an influenza B infection and elicited an immune reaction to the B virus in the ferret as well as the hamster. The remaining throat-washing was taken from a person who, following the attack, showed a rise in neutralizing antibodies to influenza A and B virus, but produced a positive response to influenza virus only in both the ferret and hamster. Thus there was complete accord in the immune reaction of the ferrets and hamsters.

Attempt was made to adapt the virus to mice after one hamster-passage with 5 of the throat-washings which had produced an immune reaction to the A virus. In each instance following 3 to 4 mouse-passages, pulmonary lesions began to appear, and on further passages the virulence increased and the mice began to die with typical pulmonary consolidation. It was subsequently shown that the agent producing these lesions was filtrable, and was neutralized by influenza A virus immune serum.

An effort to transfer from a hamster to mice the virus identified as influenza B virus by the immune response, was not successful. The failure may be attributed to the presence of a "wild virus," which seemingly had its origin in the hamster. This virus became rapidly fatal to mice, and would probably have masked an infection of influenza B virus, had the latter existed.

Discussion and Summary. The comparative inoculations which have been made in hamsters and ferrets indicate that the hamster may be substituted for the ferret in identifying influenza A virus in throat-washings, either through the production of specific neutralizing antibodies to this virus, or as the first step in adapting the virus to mice. The one throat-washing at our disposal which contained type B influenza virus, likewise produced an immune response in the hamster; but owing to a contaminating "wild virus" success was not achieved in adapting the type B influenza virus to mice after a passage in the hamster.

It should be emphasized, however, that the titer of the hamster serum following infection is usually not so high as that of the ferret and it is therefore advisable to use not more than 100 MLD of virus in performing the neutralization-test.

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A Selective Medium for Isolation of *Hemophilus influenzae.*

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The isolation of Gram negative pathogenic microörganisms from the nasopharynx is often difficult because of overgrowth by Gram positive cocci. This difficulty is increased when the organism requires an enriched medium which favors the growth of normal flora. Fleming[1] inoculated plates with penicillin and noted that Gram positive cocci could be inhibited and *H. influenzae* obtained in relatively pure culture.

The determination of the incidence of *H. influenzae* in the nasopharynx, because of its possible relation to the severity of influenza epidemics, is of considerable importance. A more convenient method of isolation than those available at present would be of obvious value. Accordingly we investigated the effect on this organism of the bactericidal agent, tyrothricin, isolated by Dubos.[2]

The tyrothricin was prepared according to the method of Dubos.[2, 3, 4] Following saline precipitation, it was kept in the form of a stock alcoholic solution (8 mg/100 cc). The tyrothricin was diluted in 5% glucose in distilled water. It was incorporated into the medium by placing 1.5 cc of the dilution to be studied in a Petri plate and adding 15 cc of Fildes' peptic blood digest agar at 45°C. The plates were allowed to dry at room temperature and 0.1 cc of inoculum spread over the surfaces. Chocolate agar plates were similarly made containing tyrothricin. A heavy inoculum of *Staphylococcus aureus,* mixed with varying dilutions of *H. influenzae* was used.

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1 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

2 Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1.

3 Dubos, R. J., and Hotchkiss, R., *J. Biol. Chem.*, 1940, **136**, 803.

4 Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.