which gave positive reactions were caused by any one of a group of several related viruses should be considered. Some of these agents may not be directly transmissible to mice.

Summary. Complement fixation tests were done with sera from cases of pneumonitis and acute upper respiratory disease and antigens from the viruses of lymphogranuloma venereum, meningopneumonitis, and the mouse pneumonia virus of Nigg.[4] Positive reactions of a group-specific character were obtained in 10 to 15% of cases of pneumonitis and in about 2% of cases of upper respiratory disease.

Most of the serum specimens tested in this study were obtained through the courtesy of Drs. W. G. Donald, R. T. Sutherland, and Henry Borson, Cowell Memorial Hospital, University of California; Drs. W. J. Kerr and J. W. Brown, University of California Medical School; and Drs. J. Thomas Hardesty, J. E. Walker, and G. B. Hanson, Seaside Memorial Hospital, Long Beach, California.

13878

Detection of Tubercle Bacillus Polysaccharides in Infected Material from Animals and Patients.*

JOHN T. RIORDAN. (Introduced by Francis G. Blake.)

From the Section of Preventive Medicine, Yale University School of Medicine.

In a previous communication[1] a series of precipitin and collodion-particle-agglutination tests has been reported with antigens derived *in vitro* from tubercle bacilli. In view of the extreme sensitiveness of some of these tests, in which positive collodion-particle-agglutination tests were obtained with the tubercle bacillus polysaccharides, in a dilution of one part in ten million, it would seem that, if these polysaccharides exist free within tuberculous lesions, they should be demonstrable there by these methods. It has been our purpose, therefore, to see whether or not this is true; so in the present communication a series of these tests which have been performed on pathological material is recorded.

Materials. A single lot of anti-H37 tubercle-bacillus serum (designated No. 5807A and B) was used as in the former series.[1] † It was prepared by the Mulford Biological Laboratories of Sharp and Dohme in 1925, by the injection of a horse with defatted tubercle bacilli. The immunological properties of this serum have been studied by a number of investigators,[2] including Heidelberger,[3] who found its antibody to be almost entirely of antipolysaccharide specificity. In our own tests,[1] this was confirmed, in so far as positive immunological reactions were obtained with polysaccharide fractions of acid-fast bacilli and with Old Tuberculin, but not with purified protein derivatives of tubercle bacilli.

In the present series of tests, the following material was used as a possible source of antigens which might react with this serum:

I. *Infected embryonic tissue.* Twenty, 9-day chick embryos were implanted with tubercle bacilli (human type), and 9 days later the gross lesions noted on the chorio-allantoic membrane were harvested in 3 eggs.[4] This material was washed in saline, minced,

* Aided by a grant from the John and Mary R. Markle Foundation.

1 Riordan, J. T., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 622.

† I am indebted in this and in the previous study[1] to Dr. B. Gerstl, of the Department of Pathology, Yale University Medical School, for a supply of this serum.

2 Thomas, R. M., and Duran-Reynals, F., *Yale J. Biol. and Med.*, 1940, **12**, 525.

3 Heidelberger, M., and Mengel, G. E. O., *J. Biol. Chem.*, 1937, **118**, 79.

4 Emmart, E. W., and Smith, M. I., *Public Health Reports*, 1941, **56**, 1277.

TABLE I.
Immunologic Tests for Derivatives of the Tubercle Bacillus in Material from "Infected" Tissue and Patients.

Material	Material treated by: Method A — No. of tests	Method A — Ptn.	Method A — C.P.A.	Special methods B and C — No. of tests	Special methods B and C — Ptn.	Special methods B and C — C.P.A.
Embryonic Tissue	3	—	—	3	+	++
Animal Tissue	10	—	—	4	+	++
Human Pleural Fluid	20	—	—	0	....	....
Human Sputum	8	—	—	10	+	++

— Negative test. + Positive test in all cases tried. Ptn. = precipitin reactions. C.P.A. = Collodion-particle agglutination.

and ground in sand and distilled water for (A) 10 to 20 min, and (B) 60 to 90 min. The ground material was centrifuged at high speed for 30 minutes, the supernatant fluid filtered through a Seitz filter, and in most instances this filtrate was concentrated to approximately 1/10 of original volume *in vacuo* at 50°-55° C.

II. *Tuberculous tissue from monkeys.* This was chosen because of the fulminating type of tuberculous lesions seen in these animals. Tuberculous lung, liver, and spleen were used. Extracts were made by grinding tissue in sand and distilled water (roughly 1 g of tissue to 10 ml of water): (A) 10 to 20 min, and (B) 60 to 90 min. The resulting emulsions were centrifuged, passed through a Seitz filter, and concentrated in much the same manner as the embryonic extracts.

III. *Pleural fluid,* with and without demonstrable tubercle bacilli, from human cases of tuberculosis. This material was centrifuged at high speed, and the supernatant fluid tested.

IV. *Sputum,* freshly collected, and containing 5 to 20 tubercle bacilli per oil immersion field by direct smear, was (A) digested with 3% NaOH at 37° C. for one-half hour. It was then centrifuged at high speed, the supernate neutralized to phenol red with HCl, filtered as above, dialyzed through a cellophane membrane‡ against distilled water at ice-box temperature, and concentrated in the same manner as the embryonic extracts; (B) whole sputum was ground in sand and distilled water for 60 to 90 min, centrifuged, passed through a Seitz filter, dialyzed through cellophane, and concentrated *in vacuo* in the same manner as the tissue extracts; and (C) whole sputum was put in a pyrex tube, stoppered, and immersed in a water bath at 56° C for one week. Smears made of the sputum at this time by the Ziehl-Neelson technique showed some pink-staining rods as well as typical red-staining tubercle bacilli. This material was centrifuged, the supernate saved, and the sediment washed with distilled water 3 times with centrifuging in between, and the supernates pooled. They were then passed through a Seitz filter, dialyzed through cellophane, and finally concentrated by heat *in vacuo* at 50°-55° C.

‡ The cellophane tubing used in these experiments is known as Visking casing, 36/32 inch in diameter.

Results listed in Table I indicate that none of the extracts which were not submitted to prolonged grinding or autolysis yielded serological evidence that tubercle-bacillus polysaccharides existed free in this material (see under columns labelled Method A). Positive results were obtained only with material subjected to Special Methods described above (see under columns labelled Special Methods B and C).

Discussion. In spite of the fact that the polysaccharide fraction of the tubercle bacillus is regarded as the main fraction most soluble in water, it is not easy to demonstrate minute traces of it in tuberculous lesions, or in fluid which may have been in contact with such lesions. Only when the pathological material was subjected to agents that may artificially disintegrate the tubercle bacilli present, did the serological tests yield evidence of the

presence of this antigen.

Conclusion. The polysaccharide fraction of the tubercle bacillus may be liberated in the course of infection produced by this organism, but such fractions are not readily demonstrated by the delicate immunologic methods used in these experiments. The positive results herein recorded have been obtained only in material treated by *artificial* agents which may have disintegrated the tubercle bacilli present.

13879 P

Pituitary and Nervous Control of Chromatic Responses, Especially of Xanthophores, in Killifish (*Fundulus*).

E. F. B. FRIES. (Introduced by A. J. Goldforb.)

From the Department of Biology, the City College, New York, and the New York Aquarium.

Previous work on killifishes has shown the yellow pigmentary effectors (xanthophores) of *Fundulus heteroclitus* and *F. majalis* to be subject to humoral control and to direct, probably double, nervous control permitting independence from melanophore responses.[1] A pituitary hormone dominates the xanthophores of the minnow *Phoxinus*,[2] as it does amphibian melanophores. Pituitary extract causes xanthophore pigment dispersal in this and other teleosts—including *Fundulus*.[3,4] The present paper reports experiments undertaken to verify and evaluate the inferred participation of the hypophysis in pigmentary coördination, with respect chiefly to the xanthophores, in *Fundulus*.

Chromatic responses were determined in 54 *F. majalis* 5 or more days after total hypophysectomy,[5] *i.e.*, when pituitary substances were absent from the circulation. Black-bottomed and white-bottomed vessels evoked darkening and paling, through melanophore change, about as readily as in normal fish (as in *F. heteroclitus*).[6] The hypophysectomized *F. majalis* were clearly incapable of normal adaptive response to yellow, however, irrespective of the duration of their stay over that color (instead of white or pale blue). Yet they became certainly, though only slightly, more yellowish after staying over yellow than otherwise. This fact was verified by reciprocal comparative tests, involving interchange of the yellow and other pale substrates. The xanthophores of the tail fin (as examined microscopically) after hypophysectomy did not effect the usual pigment-dispersing response to yellow, but they did respond to black, thus showing that pituitary intervention is not essential for eliciting their pigment dispersal.

In 20 *F. heteroclitus* the effect of total hypophysectomy on xanthophore response was greater. These fish remained uniformly grayish over a pale ground, whether yellow, white, or blue. Their caudal xanthophores showed none of the normal pigment-dispersing response to yellow (contra Matthews).[3] Nevertheless, these xanthophores could still show the temporary pigment dispersal that may be brought about by handling.

The table summarizes results of keeping normal (N) and pituitaryless (H) fish of both species several hours in bowls of the designated colors.

Pituitary involvement was so manifest in the results of hypophysectomy as to stimulate reconsideration of the question of direct nervous control of the xanthophores. To this end, one or more caudal fin rays were tran-

[1] Fries, E. F. B., *J. Exp. Zool.*, 1931, **60**, 389; *Biol. Bul.*, 1942, **82**, 261.

[2] Giersberg, H., *Z. vergl. Physiol.*, 1932, **18**, 369.

[3] Matthews, S. A., *Biol. Bul.*, 1933, **64**, 315.

[4] Abramowitz, A. A., *Am. Nat.*, 1936, **70**, 372.

[5] For method, see Abramowitz, A. A., *Science*, 1937, **85**, 609.

[6] Reviewed by Parker, G. H., *Proc. Am. Acad. Arts Sci.*, 1940, **73**, 165.