planned. We are inclined to believe that the activity after injection gives a more accurate picture of the comparable T_4-like activity of T_4 analogs in the tadpole using the tail response. Activity comparisons should also be made using other criteria, for example, limb eruption and growth (*4*).

The tadpole response after immersion is probably strongly influenced by relative rates of penetration. Absorption undoubtedly occurs through the skin and the gills, since tying off the gut did not influence the response of the tadpole to T_3. It is consistent with the classical rules of permeability that a compound with only an acetate or propionate side chain would be more permeable than a compound with a divalent alanine zwitter ion side chain. Immersion tests might also be more subject to difficulties arising from the instability of compounds (*5*) and to adsorption on glass at high dilutions (*6*). Finally, it should be added that the results may help to explain certain enhancements and inhibitions of the tadpole response in immersion experiments (*7*). The many varied and confusing results obtained in this latter area may be due to effects on the permeability of T_4 and T_3 rather than representing an effect on the peripheral tissue response to the tryroid hormone (*8*).

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References and Notes

1. For a rather complete summary of much of the data on the relative activity of T_4 analogs, see R. V. H. Pitt-Rivers and J. R. Tata, *Thyroid Hormones* (Pergamon, London, 1959).
2. Only one example is cited here, that is, E. Frieden and R. T. Flitman, *Arch. Biochem. Biophys.* **64**, 513 (1956).
3. For a recent paper in this series see A. E. Herner and E. Frieden, *J. Biol. Chem.* **235**, 2845 (1960).
4. The recent experiments of J. Kollros *et al.*, presented at the annual meeting of the American Institute of Biological Sciences, Stillwater, Okla., on 29 August 1960, suggest that certain skin and other responses may be more sensitive to extremely low doses of T_4 in the hypophysectomized tadpole. Since the tadpole was immersed in the T_4 solution, it is possible that the skin and related structures received a relatively larger proportion of the very dilute hormones used.
5. Problems regarding the stability of T_4 and analogs have been mentioned recently by W. L. Money, R. I. Meltzer, J. Young, and R. W. Rawson [*Endocrinology* **63**, 20 (1958)] and by N. R. Stasili, R. L. Kroc, and R. I. Meltzer [*ibid.* **64**, 62 (1959)].
6. N. Freinkel, S. H. Ingbar, J. T. Dowling, *J. Clin. Invest.* **36**, 25 (1957); The data of S. Lissitsky, M. Roques, and M. J. Benevent [*Biochim. et Biophys. Acta* **41**, 252 (1960)] suggest that T_4 analogs which do not have free amino groups or 3,5 iodines are not adsorbed appreciably on glass at $3 \times 10^{-8} M$, *p*H 6.4, 370.
7. E. Frieden and B. Naile, *Science* **121**, 37 (1955).
8. This work was aided by a grant, C-3006, from the U.S. Public Health Service. A report of this work was included in a symposium on metamorphosis at the AIBS meeting, Stillwater, Okla., 29 August 1960.

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Nature of "Sex-ratio" Agent in Drosophila

Abstract. Several lines of evidence implicate small spirochetes, presumably treponemata, as etiologic agents in the production of the maternally transmitted "sex ratio" condition (SR) in *Drosophila nebulosa,* in *D. willistoni,* and in strains of *D. melanogaster* into which the SR condition has been artificially transferred. The presence of treponemata in the hemolymph of adult females of these species is completely correlated with the production of unisexual progenies and like this condition is dependent on the genotype of the host and of the infectious agent.

A condition of unisexual (or near unisexual) progenies in *Drosophila* known as "sex-ratio" (SR) has been intensively studied in a number of species by several investigators (*1*) who showed it to be maternally transmitted and dependent on an agent variously interpreted as a plasma gene, cytoplasmic particulate, or virus (*2*). The pattern of transmission has been established in *D. bifasciata, D. equinoxialis, D. nebulosa, D. paulistorum, D. prosaltans,* and *D. willistoni* (*3*), and in all except the first it has been shown that the stability and persistence of the condition is also dependent on the nuclear genotype of the flies. In *D. bifasciata* and *D. equinoxialis* this condition has been shown to be temperature sensitive and subject to thermic cure (*4*). The unisexual progenies are a consequence of disturbances of development in male zygotes which lead to 50 percent egg mortality (*5*). The pattern of disturbance and the stage of onset are strongly influenced by the genotype of the zygote, and there is evidence that female as well as male zygotes may be affected (*6*).

That the "sex-ratio" agent is of an infectious nature (even though the condition is not contagious) was demonstrated in experiments in which the condition was transferred by injection of ooplasmic materials from SR strains into previously normal strains of *D. willistoni* and *D. equinoxialis* (*7*). In the course of such experimental infections the condition makes its appearance after an incubation period of 10 to 12 days in many or all of the infected females. In some instances the condition may only make its appearance in later generations after a period of latent transmission (*8*). A thorough study of the course of several such infections showed that the otherwise normal daughters of injected females may transmit a sporadic and nonspecific zygote lethality which can be carried through more than 20 generations of their descendants (*9*).

A study of the distribution of the "sex-ratio" agent in tissues and organs of SR adults of *D. willistoni* demonstrated its presence in ovary, fat body, flight muscle, and in exceptionally high concentration in hemolymph (*10*). The agent was also found in high concentration in the hemolymph of the rare surviving sons of SR females in this species. Further, the agent may be present in latent form in flies of apparently normal strains of *D. willistoni,* as shown by infections produced from injections of extracts of such flies into other females of the same strain. Attempts to separate and concentrate the infective agent of *D. willistoni* by centrifugation and ultrafiltration of extracts of flies showed that the activity (as measured by frequency of infections) of supernatant fluids from whole-fly homogenates is not reduced on passage through a Millipore filter of pore size 0.3 μ, but is cut to about one-third by passage through a Millipore filter of pore size 100 mμ (*11*).

Microscopic examination of the hemolymph of females of *D. nebulosa* and *D. willistoni* giving strictly unisexual progenies shows the regular presence of many very fine filaments which are more numerous in older than in newly emerged flies. These filaments, which are absent from the blood of normal females of both species, are in constant motion in fresh preparations of hemolymph mounted in Crown immersion oil. They are visible only with dark-field or phase-contrast microscopy and in thin preparations can be seen to be of the order of 0.1 to 0.2 μ in diameter and to average 4 to 5 μ in length, although occasional individuals may be 8 to 10 μ in length. In favorable freshly mounted preparations, waves of sinusoidal or helicoidal movement are seen to pass along the length of the filaments, giving them a regular spiral appearance. In such preparations the filaments remain visible and active up to 48 hours at 25°C, although they become increasingly granular and fuzzy-edged, and evidences of spiralizing vanish. During this time there is an increase in the number of minute granules free in the hemolymph. At the end of a week's time the hemolymph preparations contain principally such small granules, evidently derived from the filaments. The granules appear to have a minute, almost invisible, tail or flagellum and maintain an active movement distinguishable from Brownian movement. Similar small active granules are numerous in the blood of rare surviving sons of SR females. Occasional granules of this sort are encountered in the hemolymph of most normal females and males.

The most satisfactory permanent preparations have been obtained by fixation of small drops or smears of

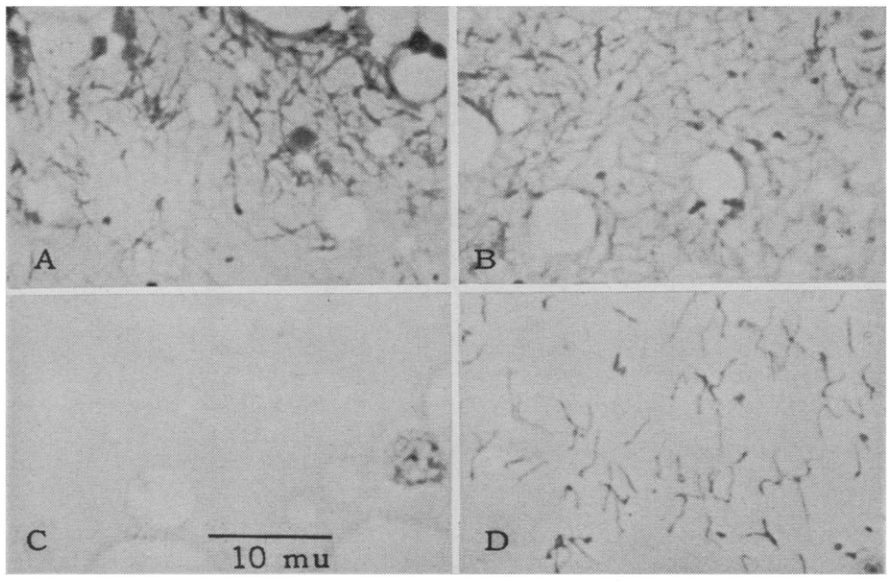

Fig. 1. Hemolymph smears from SR and normal females. Giemsa stain, phase contrast, scale for all photos is given in (*C*). (*A* and *B*) *Drosophila nebulosa*, SR strain PV-45, fixed in formaldehyde vapor, showing numerous spirochetes. (*C*) *D. nebulosa*, normal strain PV-59, no spirochetes; clear areas indicate fat droplets; small blood cell nucleus at right. (*D*) *Drosophila melanogaster*, SR strain (XXY) derived by transfer of SR from the SR B-3 strain of *D. willistoni*; fresh Giemsa preparation without fixation; spirochetes somewhat swollen and well stained.

hemolymph in formaldehyde vapor followed by one or another of the standard staining procedures (*12*). The filaments stain a strong purplish-red with Giemsa, are intensely colored by Fast Green employed as a basic protein stain, and are moderately colored by Azure B under conditions of ribonucleic acid staining. The filaments are Gram-negative, but give indications of slight Feulgen positivity. They blacken readily with Fontana's silver method. Their characteristic appearance in Giemsa preparations is seen in Fig. 1.

The filaments are sensitive to penicillin and rapidly disappear from the hemolymph of SR females injected with penicillin G. However, they reappear after a few days in the absence of further injections of penicillin. Normal hosts injected with penicillin-treated SR blood produced no SR progeny, while controls gave 100 percent SR offspring. Filaments were completely absent in the former and invariably present in the latter. The filaments have all the characteristics of small spirochetes and resemble those of the genus *Treponema* more closely than any other described forms (*13*).

Further evidence of the correlation between the "sex-ratio" condition and the presence of treponemata derives from experiments in which the condition was transferred from *Drosophila willistoni* into *D. melanogaster* (*14*). There, as in the original form, all females producing unisexual progenies possess the treponemata in their hemolymph. Where loss of the unisexual condition has occurred the spirochetes are lacking. The stability of the condition in *D. melanogaster* is dependent on genotype and, in some instances at least, on temperature.

Although apparently different from a larger spirochete described by Chatton in 1912 from *D. confusa* and named by him *Treponema drosophilae* (*15*), the rare occurrence of giant forms in the blood of very old flies (especially in *D. nebulosa*) does raise the question of a possible relationship. Since it has been demonstrated that the "sex-ratio" agent, and thus spirochetes, may be carried in latent condition, the whole subject of interrelationships of spirochetes and *Drosophila* deserves thorough investigation.

It is concluded that the "sex-ratio" condition in *Drosophila* species is a consequence of a very special, stabilized relationship between spirochete and host in which the genotypes of both are of prime importance (*16*).

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References and Notes

1. G. E. Magni, *Ist. Lombardo Sci. Lettere* **75**, 3 (1952); A. G. L. Cavalcanti and D. N. Falcão, *Caryologia* **6**, 1213 (1954); A. G. L. Cavalcanti, D. N. Falcão, L. E. Castro, *Univ. Brasil Publ. fac. nacl. fil. Ser. cient. No. 1* (1958); H. L. Carson, *Drosophila Inform. Serv.* **30**, 109 (1956); C. Malogolowkin and D. F. Poulson, *Science* **126**, 32 (1957); C. Malogolowkin, *Genetics* **43**, 274 (1958).
2. A. G. L. Cavalcanti, D. N. Falcão, L. E. Castro, *op. cit.* (1958); G. E. Magni, *Atti 2nd Riun. Assoc. Genet. Ital.* **26**, 1 (1956); *ibid.* **27**, 1 (1957); C. Malogolowkin and D. F. Poulson, *Science* **126**, 32 (1957); D. F. Poulson and C. Malogolowkin, *Proc. Intern. Congr. Zool. 15th Congr. London* (1959), p. 200.
3. G. E. Magni, *Ist. Lombardo Sci. Lettere* **75**, 3 (1952); *Atti 2nd Rivn. Assoc. Genet. Ital.* **26**, 1 (1956); A. G. L. Cavalcanti, D. N. Falcão, L. E. Castro, *Am. Naturalist* **91**, 321 (1957); *Univ. Brazil Publ. fac. nacl. fil. Ser. cient. No. 1* (1958); C. Malogolowkin, *Genetics* **43**, 274 (1958); C. Malogolowkin, *Am. Naturalist* **93**, 365 (1959); D. F. Poulson, unpublished data on *Drosophila nebulosa*, from strains kindly provided by H. L. Carson of Washington University, St. Louis, Mo.
4. G. E. Magni, *Caryologia* **6**, 1213 (1954); C. Malogolowkin, *Am. Naturalist* **93**, 365 (1959).
5. C. Malogolowkin and D. F. Poulson, *Science* **126**, 32 (1957); C. Malogolowkin, D. F. Poulson, E. Y. Wright, *Genetics* **44**, 59 (1959).
6. D. F. Poulson and S. J. Counce, *Anat. Record* **134**, 625 (1959); unpublished data.
7. C. Malogolowkin, D. F. Poulson, E. Y. Wright, *Genetics* **44**, 59 (1959); C. Malogolowkin, *Am. Naturalist* **93**, 365 (1959).
8. C. Malogolowkin, D. F. Poulson, E. Y. Wright, *Genetics* **44**, 59 (1959); D. F. Poulson, unpublished data.
9. D. F. Poulson, unpublished data.
10. B. Sakaguchi and D. F. Poulson, *Ann. Rept. Natl. Inst. Genet. Japan* **10**, 27-28 (1959).
11. ———, unpublished data.
12. R. D. Lillie, *Histopathologic Technic and Practical Histochemistry* (Blakiston, New York, 1954), p. 399; R. E. Campbell and P. D. Rosahn, *Yale J. Biol. and Med.* **22**, 527 (1950).
13. *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, Baltimore, ed. 7, 1957), pp. 896–907; T. B. Turner and D. H. Hollander, "Biology of the Treponematoses," *World Health Organization Monogr. Ser. No. 35* (1957).
14. B. Sakaguchi and D. F. Poulson, *Anat. Record* **138**, 381 (1960); D. F. Poulson and B. Sakaguchi, *ibid.* **138**, 376 (1960); C. Malogolowkin, by personal communication, informs us that the SR strain of *Drosophila equinoxialis* carries treponemata indistinguishable from those reported here.
15. E. Chatton, *Compt. rend. soc. biol.* **73**, 212 (1912).
16. The research was supported by grant No. 6017 from the National Science Foundation.

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