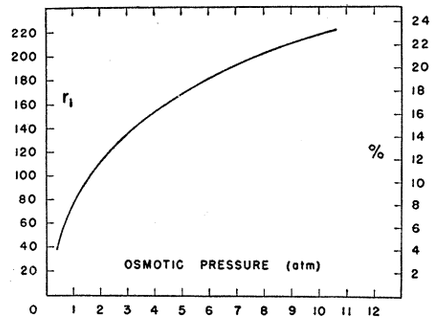

Fig. 1. The osmotic pressure of aqueous solutions of Carbowax molecular weight 20,000 as a function of the density in percent by weight, and the refractive index, r_i, of the solutions at 20°C (*12*).

lar weight—henceforth referred to as PVP, C6M, and C20M, respectively—was attempted by dialysis (*5*). Osmotically equivalent quantities of the purified products were dissolved in Hoagland's nutrient solution, which was diluted four times (Table 1). The magnitude of the aliquots taken followed from the relationship between the concentration and osmotic pressure of aqueous solutions (Fig. 1) as determined by the thermocouple psychrometer method (*6*).

The yield data indicate that nutrient solutions containing C20M produced slightly higher fresh weights of kidney beans than did isotonic solutions without C20M. This observation points to the absence of effects other than osmotic ones in the case of C20M. Less likely would be an alternative explanation implying that the C20M in the one case and the excess of ions in the other case exert an osmotic and ionic composition effect, the sum of which is of approximately equal magnitude. The lower yields, and the nonlinearity between osmotic pressure and yield, of plants grown in solution cultures containing dialyzed C6M and PVP, as compared to the yields of plants grown in isotonic solutions containing ions only, point to the physiological toxicity of these compounds.

An efflorescence of white material appearing on the upper surface of leaves of kidney beans grown in solutions containing C20M was shown by infrared analysis (*4*) to be identical to C20M. This finding suggests that C20M had passed through the plant system without undergoing any breakdown of its basic structural unit. Comparable transport of macromolecular substances through plant systems has been reported (*7*). These considerations, in conjunction with the existing knowledge on the structure of polyethylene glycols, render it improbable that C20M per se might exert any physiological effect on plant growth. The results of a more comprehensive experiment (*8*) support the viewpoint that purified C20M is physiologically inert to the extent that it can be used to control the osmotic pressure of plant culture solutions pending the effect of any interaction between ions and C20M.

Observations were made pertinent to this interaction. For this purpose, both ions and C20M were dissolved in concentrations equivalent to 1, 2, and 3 atm of osmotic pressure (Table 2). With the Beckman No. 78178V sodium selective electrode (*9*), the mean activity of sodium chloride was measured in the presence and absence of C20M, and mean activity coefficients were calculated. The results indicate that an increase in the concentration of C20M raises the mean activity coefficients of sodium chloride to well above unity. It is postulated that the large C20M molecules participate in a process of solvation. The ensuing arrangement of water molecules would accord a greater activity to the cations present. The occurrence of solvation would also explain why aqueous solutions of C20M do not observe Van't Hoff's law, complicating the freezing point depression method as a way of ascertaining the osmotic pressure of the solutions (see *10*).

The interacting effect of C20M on cations is, of course, mutual. This becomes at once clear from a comparison of the results of osmotic pressure measurements of mixed solutions of ions and C20M with the results of the corresponding separate solutions (Table 2). As the ionic concentrations were increased, the measured osmotic pressure of the ionic solutions of C20M appeared to be considerably in excess of the sum of the osmotic pressures of the separate solutions of C20M and ions. Under the same circumstances, the relative viscosity of the solutions decreased slightly for any one concentration of C20M. Both phenomena may be explained on the basis of an apparent dissociation of the long-stretched C20M molecule into shorter molecules of the same basic structure under the influence of ions, the decrease of the particle size of the solute being accompanied by an increase of the particle density of the solution. This feature complicates the use of C20M for controlling the osmotic pressure of plant nutrient solutions (*11*).

J. V. LAGERWERFF
GEN OGATA
HAROLD E. EAGLE
U.S. Salinity Laboratory, Riverside, California

References and Notes

1. Union Carbide Chemicals Co.
2. E. I. Du Pont de Nemours and Co.
3. Antara Chemicals.
4. We are indebted to Alton W. Specht, Spectrochemical Laboratory, Agricultural Research Service, U. S. Department of Agriculture, and R. T. Ambrose, Department of Chemistry, University of California at Riverside, for the spectrographic and infrared analyses, respectively, of samples of Carbowax.
5. Sacks prepared from cellulose casing, 5½ in. (inside diameter) (Visking Corp.) were filled with about 5 liters of a solution of the osmotic agent in water and suspended in running tap water for 10 days. Deterioration of the sacks due to chemical corrosion necessitated the exercise of extreme care in collecting the dialyzed solution.
6. L. A. Richards and G. Ogata, *Science* **128**, 1089 (1958).
7. H. Groenewegen and J. A. Mills, *Australian J. Biol. Sci.* **13**, 1 (1960); J. W. Mitchell, I. R. Schneider, H. G. Gauch, *Science* **131**, 1863 (1960); W. A. Jensen and A. D. McLaren, *Exptl. Cell Research* **19**, 414 (1960).
8. J. V. Lagerwerff and G. Ogata, "Plant growth as a function of interacting activities of water and ions under saline conditions," *Intern. Soc. Soil Sci. Proc.*, in press.
9. C. A. Bower, *Soil Sci. Soc. Am. Proc.* **23**, 29 (1959).
10. H. G. Applegate, *Nature* **186**, 232 (1960). The freezing point depression method yields values which are from 20 to 40 percent higher than those indicated by a thermocouple psychrometer for solutions ranging from 1 to 8 atm osmotic pressure.
11. This report is a contribution from the U.S. Salinity Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, U.S. Department of Agriculture, Riverside, Calif., in cooperation with the 17 western states and Hawaii.
12. The relationship shown to exist between the osmotic pressure and the density of C20M solutions is accurately reflected by their refractive index, r_i. Measurements have shown that, for all practical purposes, the influence of ions and of temperature on the r_i values of C20M solutions is negligible. Therefore, r_i measurements constitute a simple and fast reference method for obtaining the osmotic pressure value of ionic solutions of C20M, for as far as due to this compound.

18 November 1960

On the Anomalous Activity of Thyroxin Analogs in Tadpoles

Abstract. The abnormally high thyroxin-like activity of certain thyroxin analogs is due to the use of the unique test route of immersion. When thyroxin, triiodothyronine, and their propionic and acetic acid analogs are tested by injection into the tadpole, their activities are more comparable to activity levels established in rat and man.

It has been frequently reported that certain side-chain variants of thyroxin (T_4) and triiodothyronine (T_3) showed many times the activity of thyroxin when compared for their ability to induce metamorphosis in anura (*1*). For example, the compound, 3,5,3′-triiodothyropropionic acid has been variously reported to be 200 to 300 times as active as T_4. However, the abnormally high activity of these T_4 analogs does not appear in T_4 activity comparisons in other in vivo or many in vitro systems (*1*, *2*). Thus the quantitative aspects of the tadpole response to certain T_4 analogs seemed to be unique.

Our continuing interest in the role of T_4 in general, and the biochemical aspects of anuran metamorphosis in par-

Table 1. Comparative activity of thyroxin and analogs. Data on rats, taken from Pitt-Rivers and Tata (*1*), are based on the oxygen uptake and goiter prevention tests.

Compound	Activity compared with thyroxin			Relative activity, injection/ immersion †
	Rat (injection)	Tadpole		
		Rana grylio injection	*Rana* spp* (immersion)	
Thyroxin (T_4)	1.0	1.0	1.0	17
3,5,3′,5′-Tetraiodothyropropionic acid	0.1–0.6	3	21–100	1
Triiodothyronine (T_3)	1–10	17	5.0–20	‡
3,5,3′-Triiodothyropropionic acid	0.1–0.5	7	15–300	0.4
3,5,3′-Triiodothyroacetic acid	0.3–1.5	7	10–24	3

* Summary of experiments with several species, including *R. grylio*, *R. clamitans*, *R. catesbeiana*, and *R. pipiens*, taken from this report and from Pitt-Rivers and Tata (*1*). † Calculated from data as in Fig. 1. Considerable experimental variation was observed in this ratio. ‡ About 100.

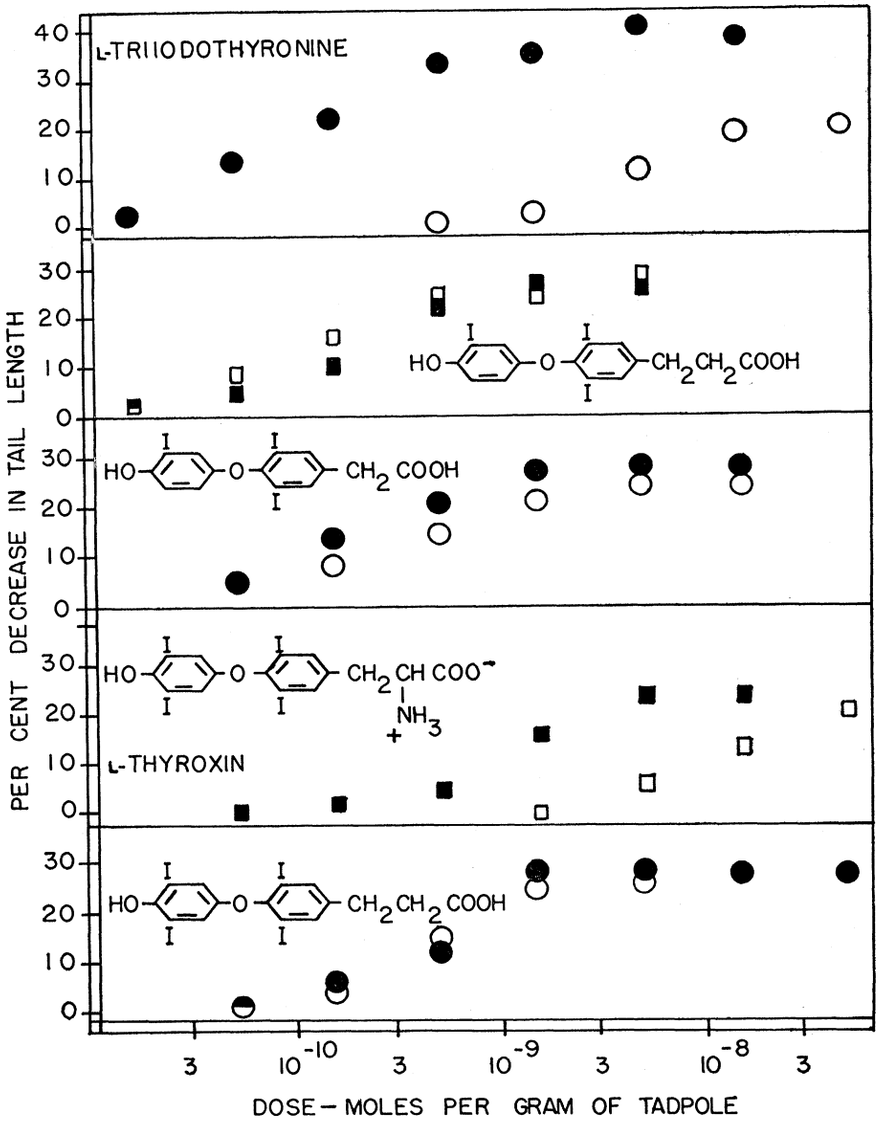

Fig. 1. Comparison of the activity of triiodothyronine, thyroxin, and analogs in *Rana grylio* after injection (filled symbols) and immersion (open symbols). The percentage decrease in tail length was measured after 5 days at 24°±1°C. The horizontal scale is a log-scale of the moles of compound given per gram of tadpole. In the immersion experiments the indicated dose of analog per gram of tadpole was dissolved in 500 ml of redistilled water containing 0.010*M* NaCl.

ticular (*2*, *3*), has prompted a reexamination of this question. Our conclusion is that the unusual activity of T_4 analogs in tadpoles arises from an unusual testing method—immersing the tadpoles in a solution of the test compound instead of the injection of the test compound. When the test compounds are administered to the tadpole by intraperitoneal injection, the relative activity of the various T_4 analogs is comparable to the in vivo activities found in rat or man.

Relative activities for five compounds tested by both immersion and injection in *Rana grylio* tadpoles are presented in Fig. 1. The most active compound is seen to be T_3 when injected, exceeding the activity of 3,5,3′-triiodothyropropionic acid and any of the other compounds tested. The activity of these compounds in *R. grylio* after immersion is in general agreement with previously reported data on other species (*1*). The most interesting feature of the data in Fig. 1 is the great disparity between activity of both T_3 and T_4 when tested by both routes. On the other hand, triiodo- and tetraiodothyropropionic acids show virtually the same activity regardless of the mode of administration. The activity of triiodothyroacetic acid is slightly higher after injection.

Similar data, not presented here, were obtained with two varieties of *Rana pipiens* by using the percentage decrease in tail height as the morphological criterion. The divergence between the activity of T_3 when tested by injection and immersion was not quite as large, but was still highly significant. It is important to note that the disparity between responses to T_3 and T_4 with the two routes of administration remains despite some variation in tadpole response due to seasonal and possibly other difficult to control environmental factors.

The response of the three different animals used in evaluating the five compounds is summarized in Table 1. It is clearly indicated that the tadpole immersion test yields the only unique data. When these compounds are injected, their activity is essentially comparable regardless of the test animal used. In the last column, the ratio of activity resulting from injection and immersion necessary for a 20 percent decrease in tail length is estimated from the data in Fig. 1. The figures vary from about 100 for T_3 to less than 1 for the propionic acid analog.

We are currently exploring the implications of the wide difference in the immersion and injected response to T_3 and T_4 by injection and immersion. Tracer experiments with labeled T_3, T_4, and propionic acid analogs are

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*).

EARL FRIEDEN
G. WAYNE WESTMARK
Department of Chemistry, Florida State University, Tallahassee

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.

6 February 1961

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