WW convert-males with the three possible types of females are represented in the Figure, *combinations 7–9*. Actually, the following is the way of procedure.

First, the assumption that the offspring of *combination* 4 contain about 25% larvae of WW constitution was ascertained by the above mentioned breeding test with ZZ males. It resulted in the identification of 17 WW females and 37 ZW females. Implanting testes into 120 undifferentiated larvae we could therefore expect that among them about 30 were of the WW constitution, 60 of ZW, and 30 of ZZ. The mortality was 15%. In a considerable number (46) it was early recognized that the testes failed to arrest severely ovarian development, obviously because of poor takes of the grafts. All these were discarded, as were also another 23 which showed no trace of testicular development at the age of maturity (22 months). Of the remaining 32 males the progeny tests prove that 19 are ZZ males, 12 are ZW convert-males and only 1 is a WW convert-male. Another animal with male external characters proves to be a hermaphrodite. Though it copulates vigorously with stimulated females, it cannot fertilize eggs, probably because no patent efferent ductules have become established.

At the present it is still impossible to explain the relatively much too low number of WW convert-males. But this first case of its type furnishes already full proof that by the method of testicular implants also WW larvae can be induced to develop testes. This evolves from the following breeding record of this animal.

Combination 9
(male WW × female ZZ)
first offspring: 24 females (100%)
second offspring: 89 females (100%)

Combination 7
(male WW × female WW)
third offspring: 146 females (100%)
fourth offspring: 84 females (100%)

Discussion. The general importance of the fact that all three possible chromosome-gene combinations (ZZ, ZW, WW) can develop and become fertile as females as well as males will be discussed in a forthcoming paper that deals with the chromosomes of Xenopus[5]. The ease with which one can now breed one-sexed cultures and be sure of normal sex, male or female, at any stage from egg to metamorphosed frog, calls for a reinvestigation of the embryology of gonad differentiation. There will no longer be any guesswork about the earliest testicular and ovarian stages. More important, experimental work on sex changes will no longer have to rely on statistics. It is a fortunate circumstance that Xenopus can so easily be induced to breed under laboratory conditions and may be raised to and maintained at maturity with a minimum of caretaking.

Zusammenfassung. Xenopuslarven mit genetischer WW-Konstitution können durch Implantation von Hoden junger Fröschchen vermännlicht werden. Ein erstes WW-Männchen gab zweimal völlig weibliche Nachkommenschaften mit ZZ-Weibchen und desgleichen mit WW-Weibchen. Alle drei möglichen diploiden Kombinationen der Geschlechtsfaktoren sind jetzt als Männchen sowohl als Weibchen bis zur Geschlechtsreife gezüchtet und als fortpflanzungsfähig befunden worden.

K. Mikamo and E. Witschi

Anatomisches Institut, Universität Basel (Switzerland), August 11, 1964.

[5] K. Mikamo and E. Witschi, in preparation.

The Action of Iodine upon Thyroidectomized Frog Larvae

In 1919 Swingle[1] found that feeding a mixture of wheat flour and iodine could induce metamorphosis of thyroidectomized frog larvae which otherwise would not have metamorphosed. He suggested that iodine itself functioned as the hormone. Dvoskin[2] has also found that elemental iodine can act like thyroxine in the rat and chick. The growth of thyroidectomized rats can be stimulated by high concentrations of iodide, and Evans et al.[3] suggested that iodide has thyroxine-like activity.

Another explanation for the activity of iodine and iodide is that they could be converted to thyroxine *in vitro* or in the tissues of a thyroidectomized organism. Thyroxine could not be detected in thyroidectomized frog larvae incubated in radioactive sodium iodide[4], but the failure to detect thyroxine formation does not mean that it is absent. Lipner and Hazen[5] found that iodide or iodine-treated flour induces metamorphosis of thyroidectomized larvae of *Rana grylio*, but chromatography of aqueous extracts of the treated flour revealed the presence of thyroxine. It is known from the work of Ludwig and von Mutzenbecher[6] that incubation of iodine, casein and sodium bicarbonate at 37°C, pH 7–9, followed by alkaline hydrolysis, allows the detection of thyroxine, diiodotyrosine and monoiodotyrosine. These results imply that thyroxine may be formed *in vitro*, and that it is the compound that promotes metamorphosis of thyroidectomized larvae.

One purpose of this investigation was to feed iodine to thyroidectomized larvae of *Rana pipiens*, then incubate

[1] W. W. Swingle, J. exp. Zool. *27*, 1 (1919).
[2] S. Dvoskin, Endocrinology *40*, 334 (1947).
[3] E. S. Evans, A. Taurog, A. A. Koneff, G. C. Potter, I. L. Chaikoff, and M. E. Simpson, Endocrinology *67*, 619 (1960).
[4] R. A. Flickinger, Gen. and comp. Endocrin. *3*, 606 (1963).
[5] H. Lipner and S. Hazen, Science *138*, 898 (1962).
[6] W. Ludwig and P. von Mutzenbecher, Z. physiol. Chem. *258*, 195 (1939).

them in NaI^{131} and assay for thyroxine by radiochromatography. The second aim was to employ chromatographic methods to ascertain if thyroxine or diiodotyrosine was formed in the iodine-flour mixture *in vitro*, as shown by LIPNER and HAZEN[5].

Experiments with thyroidectomized larvae. Fifty larvae were thyroidectomized at stage 21[4] and then raised on a diet of boiled lettuce for two months. At this time several larvae with hind limbs over 2 mm in length were discarded, since they had not been completely thyroidectomized. Half of the remaining larvae were fed a diet of iodine-pablum and the other half received only pablum, a high protein baby cereal. The iodine was finely pulverized and mixed with pablum in a ratio of 1/100 in water, ground with a mortar and pestle and then spread upon a glass plate to dry. This schedule of feeding was continued for a period of $3^1/_2$ months; the water was changed daily. By this time the thyroidectomized larvae fed iodine were undergoing metamorphosis, while those fed pablum were not. The stage of development, total length and hind limb length of thyroidectomized larvae that were used for an incubation with NaI^{131} are given in the Table. These larvae were placed in 200 ml of tap water containing 25 μc/ml of NaI^{131} for two days. Bile was chromatographed directly, while livers were first hydrolyzed with pancreatic enzyme, extracted with butanol, chromatographed in collidine-H_2O-NH_3 and counted with an autoscanner according to previously used methods[4]. Monoiodotyrosine, diiodotyrosine and thyroxine were always added as carriers and their location detected by spraying with diazotized sulfanilic acid[7]. Neither thyroxine nor diiodotyrosine were detected in the radiochromatograms of bile and liver from the iodine-pablum and pablum-fed thyroidectomized larvae.

In vitro experiments. 10 g of pablum was suspended in 30 ml of tap water containing penicillin and streptomycin, digested with an equal volume of 2% pancreatic extract (Viokase) at pH 8.4[4], the pH was adjusted to 3.0 and extracted with 60 ml of butanol which was then evaporated to about 4 ml in a rotary evaporator. Various amounts of this extract were chromatographed in collidine-H_2O-NH_3 and components were visualized with the diazotized sulfanilic acid spray reagent. When 200 γ of monoiodotyrosine and diiodotyrosine were added as carriers, the monoiodotyrosine component had the same Rf as that obtained from the digest of the pablum. Two similar experiments were performed with 10 g lots of pablum each containing 100 mg of finely pulverized iodine. In one case the sample was incubated at 37°C for one day and then hydrolyzed, while in the other it was digested immediately with the pancreatic digest. Digestions and extractions were similar to those used for the pablum sample. The butanol extracts were evaporated to 4 ml, and various amounts were chromatographed in collidine-H_2O-NH_3. In each experiment there were two components, with Rf values of 0.168–0.175 and 0.088–0.097. Addition of 200 γ of carrier monoiodotyrosine and diiodotyrosine did not reveal any additional components and the more intense coloration due to carrier coincided exactly with the position of the two components from the hydrolyzed iodine-pablum mixtures. The low Rf values of monoiodotyrosine and diiodotyrosine are attributed to the large amount of material in the concentrated butanol extracts. The similar Rf values of the carrier and experimental components are the best evidence for the presence of both monoiodotyrosine and diiodotyrosine in the iodine-pablum mixtures.

The presence of diiodotyrosine in these digests of iodine-pablum implies that it is the active factor that promotes metamorphosis of thyroidectomized larvae. However, at an alkaline pH a small amount of conversion of diiodotyrosine to thyroxine can occur *in vitro*[9], and it is probable that thyroxine formed in this manner causes metamorphosis of thyroidectomized larvae[4]. It is quite possible that traces of thyroxine were formed *in vitro* in water extracts of iodine-wheat flour[5], but hydrolysis of the total iodine-pablum mixture in the present experiments revealed only diiodotyrosine and monoiodotyrosine[10].

The effect of feeding iodine to thyroidectomized larvae

Iodine-fed larvae	TAYLOR-KOLLROS[8] stage	Total length mm	Hindlimb length mm
1. Fed iodine-pablum for $2^1/_2$ months	XII	68	9
2.	XI	73	8
3.	XII	71	12
4. Fed iodine-pablum for $3^1/_2$ months	XIX	60	20
5.	XIX	52	12
Pablum-fed larvae			
1. Fed pablum for $2^1/_2$ months	VI	68	2
2.	VII	73	2
3.	VII	67	2
4. Fed pablum for $3^1/_2$ months	VIII	77	2.5

Résumé. L'alimentation des larves de grenouille sans thyroïdes avec un mélange d'iode et de farine (Pablum) a provoqué la métamorphose, ce qui confirme les investigations de SWINGLE[1], aussi LIPNER et HAZEN[5]. La radiochromatographie de la bile et du foie hydrolysé, après immersion des larves dans NaI^{131}, ne révéla pas la présence de thyroxine ou diiodotyrosine. La chromatographie de l'iode-farine après hydrolyse, fit apparaître de la monoiodotyrosine et de la diiodotyrosine, mais après hydrolyse de la farine, on trouva seulement de la monoiodotyrosine. Il est probable que la thyroxine, synthétisée *in vitro* à partir de la diiodotyrosine, le pH étant alcalin, fut le facteur qui déclencha la métamorphose des larves sans thyroïdes.

R. A. FLICKINGER

Department of Biology, State University of New York, Buffalo (New York USA), August 21, 1964.

[7] A. TAUROG and I. L. CHAIKOFF, in *Methods in Enzymology* (S. P. COLOWICK and N. O. KAPLAN, Eds., Academic Press, New York), vol. 4, p. 856.

[8] A. C. TAYLOR and J. J. KOLLROS, Anat. Rec. *94*, 7 (1946).

[9] C. R. HARRINGTON and R. V. PITT-RIVERS, Biochem. J. *39*, 157 (1945).

[10] *Acknowledgment.* This investigation was supported by a research grant (GB 1665) from the National Science Foundation.