

melanoma¹², Ehrlich ascites mouse tumors¹³, and in FRIEND's mouse leukemic material¹⁴.

The similarity of the virus-like particles revealed in various neoplasms of mice is reminiscent of earlier observations of viral particles in chicken neoplasms¹⁵. It might be hypothesized that these virus-like bodies are present as an inactive ubiquitous agent in mice and are acquired fortuitously as a non-specific contaminant in spontaneous or experimentally induced tumors, or vicariously during numerous serial tumor transplantations. THIERY et al.¹¹ have suggested that these structures may be a result rather than the cause of the neoplastic reaction. It might perhaps be postulated that these particles are identical, the nature and localization of the neoplastic process being determined in part by host responses. Alternatively, these bodies may represent distinct entities, indistinguishable by electron microscopy, and each producing specific neoplasms. Substantiation of any of the proposed hypotheses will require more extensive investigation into a variety of mouse tumors.

Hypervitaminosis in an Insect Larva

It is believed that B-vitamins in high concentrations may have adverse effects on the metabolism of an organism¹. Not much is known about the effect of administering higher concentrations of B-vitamins in the case of insects. The observations on the effect of feeding the larva of rice moth, *Corcyra cephalonica* (Stainton) with higher doses of biotin, nicotinic acid and riboflavin are presented here.

Corcyra larva has been successfully grown on a basal diet consisting of casein, dextrose, cholesterol, McCollum-Davis salt mixture No. 185, a mixture of B-vitamins and linoleic acid². It has been shown that thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, choline chloride, *p*-aminobenzoic acid, folic acid and biotin are essential for the normal growth of the larva, the latter, however, being needed only when linoleic acid is withheld from the diet². The diet used in the experiment on the administration of graded doses of biotin did not, therefore, contain linoleic acid. Table I shows the results of the test on the effect of graded doses of biotin on the growth of the larva. It is apparent that the higher concentrations of this vitamin have adverse effects on growth as indicated by decrease in weight.

The response of the larva to the administration of nicotinic acid (Table II) beyond a level of 100 µg/g of diet is suggestive of some disturbance in growth, though this

Tab. I. Growth of *Corcyra* larva on diets containing graded doses of biotin

Amount of biotin (in µg/g)	Average weight (in mg) of larva on			Percentage of survival
	12th day	19th day	26th day	
0.00	3.3	7.8	16.4	40.0
0.25	6.1	23.7	32.6	46.6
0.50	5.3	22.8	30.8	40.0
1.0	5.7	24.5	34.1	46.6
2.0	4.8	14.5	29.6	50.0
5.0	5.3	16.3	22.2	40.0
10.0	5.6	20.4	24.4	30.0
25.0	5.0	16.4	23.2	30.0
50.0	5.8	17.8	22.9	40.0

Zusammenfassung. Elektronenmikroskopische Untersuchungen des Cloudman-S-91-Melanomgewebes ergaben das Vorhandensein virusähnlicher Teile in der Kultur, die sich strukturell nicht unterscheiden lassen von den in Mäuse-Mammatumoren beobachteten. Die inneren und äusseren Gewebsteilchen waren morphologisch mit verschiedenen Mäuseneoplasmen vergleichbar.

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¹² A. J. DALTON and M. D. FELIX, Ann. N.Y. Acad. Sci. **63**, 1117 (1956).

¹³ R. A. ADAMS and A. F. PRINCE, J. biophys. biochem. Cytol. **3**, 161 (1957).

¹⁴ E. DE HARVEN and C. FRIEND, J. biophys. biochem. Cytol. **4**, 151 (1958).

¹⁵ L. DMOCHOWSKI, Cancer Res. **20**, 977 (1960).

¹⁶ This investigation was supported by research grants C-5884 and C-5585 from the National Institute of Health, Public Health Service.

Tab. II. Growth of *Corcyra* larva on diets containing graded doses of nicotinic acid

Amount of nicotinic acid (in µg/g)	Average weight (in mg) of larva on			Percentage of survival
	12th day	19th day	28th day	
0.0	0.5	1.0	1.0	6.6
2.5	5.6	11.6	16.4	40.0
5.0	6.4	25.3	34.3	46.6
10.0	6.6	28.4	34.1	56.6
15.0	6.1	25.5	pupated	46.6
25.0	7.7	23.8	pupated	53.3
30.0	6.8	29.0	pupated	50.0
50.0	6.2	28.5	33.0	56.6
75.0	6.1	30.1	40.2	50.0
100.0	2.9	24.4	41.1	46.6
150.0	3.4	23.3	40.0	53.3
200.0	2.9	21.0	42.5	60.0

Tab. III. Growth of *Corcyra* larva on diets containing graded doses of riboflavin

Amount of riboflavin (in µg/g)	Average weight (in mg) of larva on			Percentage of survival
	12th day	20th day	29th day	
0.0	5.8	15.1	34.8	53.3
2.5	6.0	16.6	pupated	53.3
5.0	5.9	18.2	36.9	50.0
10.0	6.1	15.4	32.5	56.6
15.0	6.9	17.5	36.2	50.0
20.0	6.0	16.6	34.2	46.6
25.0	6.4	18.8	32.7	50.0
30.0	6.5	18.0	34.8	50.0
40.0	5.9	17.2	30.4	46.6
50.0	4.8	16.6	pupated	43.3
75.0	3.7	14.5	17.2	40.0
100.0	3.2	15.3	18.0	43.3

¹ R. J. WILLIAMS, R. E. EAKIN, E. BEERSTECHE, and W. SHIVE, Amer. chem. Soc., Monograph No. 110 (1950).

² N. K. UBEROI, Ph. D. Thesis, Delhi University (1959).

disturbance lasts for the first two weeks. Riboflavin, too, at higher concentrations exerts deleterious effects on growth (Table III). The exact dosage at which riboflavin becomes toxic could not be determined because the casein used in the diet was not made free from this vitamin².

The present investigations reveal that some of the B vitamins at higher concentrations become toxic to *Corcyra* larva – a fact observable in many higher animals³. (A larger quantity of biotin also has a toxic effect on *Tribolium confusum*⁴). The phenomenon of the mode of action of these vitamins is being investigated.

Résumé. La biotine et la riboflavine, fortement concentrées, administrés à la larve de la phalène du riz,

Corcyra cephalonica, sont toxiques. Les hautes concentrations de l'acide nicotinique n'ont des effets défavorables que sur la croissance des plus jeunes larves de l'insecte.

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Department of Zoology University of Delhi (India), August 14, 1961.

³ H. MOLITOR and GLADYS A. EMERSON, *Vitamins and Hormones* 6, 69 (1948).

⁴ G. FRAENKEL and M. BLEWETT, *Biochem. J.* 37, 686 (1943).

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Regeneration in Isolated Tails of *Xenopus* Larvae¹

Normally regenerating tails of *Xenopus* larvae possess a good blood supply. In connection with TSCHUMI's² experiment which revealed a correlation between blood supply and limb development, it was of interest to investigate whether or not a similar relationship may be found in tail regeneration. The culture of isolated tail tips with abolished blood circulation proved to be the method of choice. Similar attempts to culture isolated tails of *Xenopus* larvae have already been made by SHAFFER³ for other purposes. In the present paper, a short account of our preliminary results will be given.

From a variety of nutrient-free culture media, the best results were obtained with Holtfreter solution⁴. In order to avoid bacterial infections we later added 0.05% sulfathiazol (Geigy). Prior to amputation the larvae were thoroughly rinsed with glass distilled water, and then transferred into aqueous solution of 0.05% sulfathiazol for 24 h. The isolated tail tips were kept individually in small Petri dishes, containing 5 ml of culture medium at constant temperature (18°C). Since they were lying close to the surface of the culture medium, sufficient supply of oxygen was provided. Under these conditions it was possible to suppress mortality almost completely and to keep even whole tails alive. Due to the absence of blood circulation, the supply of nutrient material is of course reduced, thus allowing isolated tails to survive only for a limited period of time. In a first experiment in which 7 mm of the tail tips of *Xenopus* larvae, measuring 30 mm in length, were amputated, the following times of survival were observed in the absence of sulfathiazol. From a total number of 12 tail tips, 3 died until the 6th day after amputation, the remaining 9 specimens survived, but showed some distortions, and the muscle tissue gradually became transparent. Between the 30th and the 44th day after amputation these tail tips died. It is likely that tissues are partially absorbed. According to our present experience, however, also whole tails, measuring 20 mm in length, survived in good condition for at least one month.

The vitality of isolated tails is best illustrated by (1) the persistence of muscular contraction, even resulting in displacement of the tails, and (2) in the formation of a *regenerate* at the (proximal) site of amputation. In isolated tail tips, measuring 7 mm in length, the maximum of regeneration is obtained on the average 15 days after amputation. The regenerate is then about 1 mm long (Figure 1A). In contrast, the tails of the tadpoles from which these tips were amputated, and which have a normal blood supply, produced within 15 days regenerates of 4.0 to 4.5 mm in length (Figure 1B). The histological analysis

reveals a limited capacity of tissue regeneration in isolated tails. Thus the *epidermis* regenerates rather well, but shows irregular proliferations and vesicles at the site of amputation. In the regenerate, *notochord* as well as *neural tube* differentiate almost normally, although they are considerably reduced in size (Figure 2 and 3). In addition, apparently new *blood capillaries* may be found in the regenerate, even containing blood cells, whereas in the original tail tip these same elements are subject to degeneration. The behaviour of the *muscle* cells is still obscure, since we have no evidence as yet that myoblasts

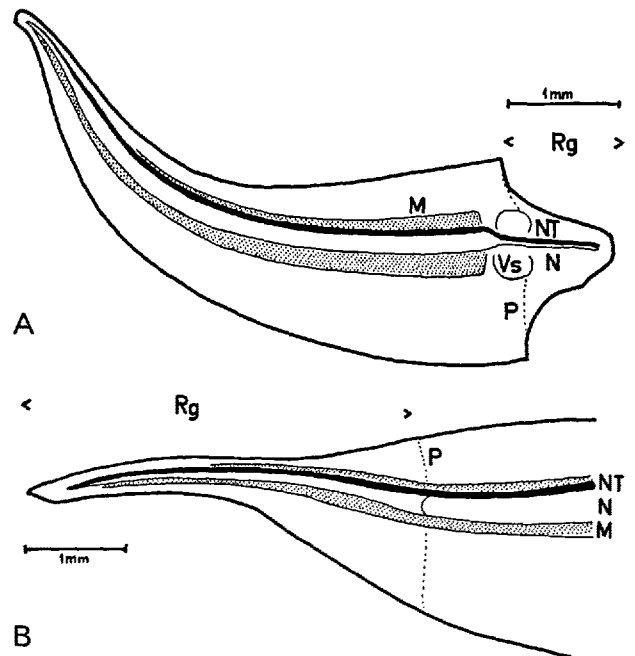


Fig. 1. (A): Isolated tail tip (7 mm), 15 days after amputation, with small heteropolar regenerate.

(B): Normal homopolar tail regeneration of a *Xenopus* larvae, 15 days after amputation (removed 7 mm).

M = muscle, N = notochord, NT = neural tube, P = pigment boundary, Rg = regenerate, Vs = vesicles.

¹ Supported by funds from the Swiss National Foundation.

² P. TSCHUMI, *J. Anat.* 91, 149 (1957).

³ B. M. SHAFFER, *Demonstration at the IXth Internat. Congr. for Cell Biology*, St. Andrews (1957).

⁴ F. E. LEHMANN, *Einführung in die physiologische Embryologie* (Birkhäuser Verlag, Basel 1945).