It has been shown that oxidation-reduction reactions occur in orbitals belonging to the ring of free base porphyrins, but in metal centered orbitals of the metalloporphyrins[14]. These considerations suggest that attention be directed towards study of TPPS or similar compounds radioisotopically labeled directly within the ring structure[15].

Résumé. Des résultats antérieurs suggèrent que l'accumulation sélective de TPPS au niveau de certaines tumeurs est liée aux caractères particuliers de leur métabolisme. Au cours d'expériences utilisant des préparations tissulaires, il n'a pas été possible de mettre en évidence une dégradation du TPPS. D'autres mécanismes impliqués dans la distribution du TPPS sont discutés.

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Indigenous Microbial Flora and the Large Intestine in Tadpoles

In terrestrial vertebrates, the large intestine is the site of marked bacterial proliferation, whereas the contents of the stomach or small intestine appear to be sterile or sparsely populated with bacteria[1-3]. The mechanical cleansing of the lumen by continuous motor activity, aided by mucus secretion, seems the most important mechanism to keep the small intestine free from debris and bacteria; in contrast, debris accumulate and many microorganisms fluorish in the large intestine, where movements are sluggish and mucus is largely disimbibed or demolished[4,5]. In fish, on the contrary, the hindgut is not enlarged[6,7], the faeces present large quantities of mucus[8] and the intestinal flora appears to be absent or restricted to a very scanty and labile form[1].

The finding that a true indigenous microbial flora is associated with the hindgut of adult amphibia[1-3] and the consideration that the larval state seems to be an old inheritance of the amphibia, handed down from their fish ancestors, prompted us to investigate if stable bacterial populations are established in the intestines of tadpoles.

Larvae from different species (*Rana esculenta, R. temporaria, Bombina variegata, Bufo viridis*), in stages from 24–29 (according to WITSCHI[9]), were examined for the intestinal bacterial content, using techniques previously described[1,2]. Full evidence was obtained that, in the hindgut of tadpoles, a multiform microbial flora is firmly established, that it continues to fluorish even after fasting periods of 1–2 weeks, ultimately being handed down, through the metamorphosis, to the adult hosts.

Are tadpoles provided, then, with an 'enlarged hindgut'? We were not able to find in the literature adequate references on this point. But, from our dissections, it became evident that tadpoles have a very well developed 'large intestine': this (Figure 1), 10–15 mm long and 1–1.8 mm in diameter, has an elongated, pyriform profile;

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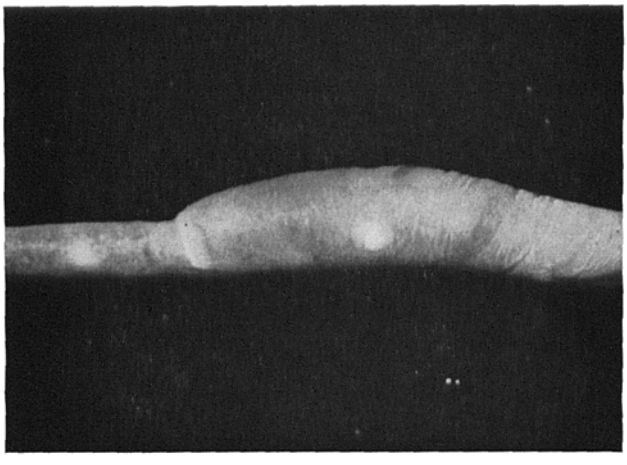

Fig. 1. The 'large intestine' of a tadpole of *Rana esculenta*, stage 25. × 10.

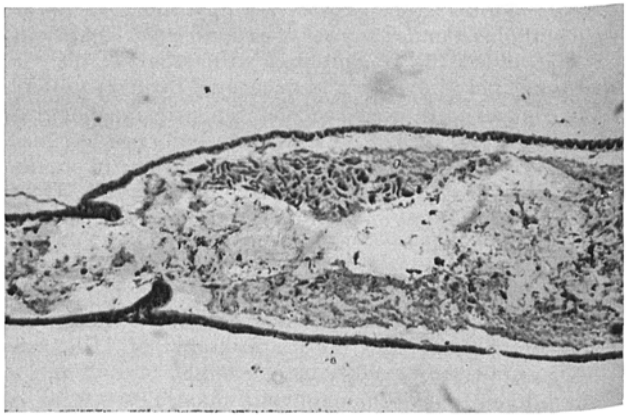

Fig. 2. Same specimen of Figure 1, logitudinal section. From left to right: end of the 'small intestine'; valve; 'large intestine' showing the central mucus flow and the characteristic agglomerate of opalinids. × 25.

the enlargement begins rather abruptly on the side of the 'small intestine', but gradually diminishes toward the rectum. It shows circular contraction waves, running briskly in a peristaltic or even antiperistaltic direction. There is (Figure 2) a flap-like valve with sphincter, similar to that found in the adult forms and seemingly homologous to the ileo-caecal valve of higher vertebrates. The flow of the contents, rich with water and mucus, pushed from the small into the enlarged intestine, is often clearly visible: the main flow streams centrally, whereas the flow close to the walls is slowed down and probably reversed and the liquid is disimbibed. This kind of 'turbulence' can probably explain why colonies of opalinids and microorganisms are characteristically located in a tract of the lumen near the wall, at a short distance from the valve. Perhaps the presence of this localized reservoir of parasites and commensals corresponds, functionally at least, to some primitive form of the caecum.

Riassunto. Nell'intestino posteriore dei girini alberga una flora microbica stabile, simile a quella degli Anfibi adulti. Ciò ben si accorda col reperto di peculiari aspetti morfo-funzionali dell'intestino posteriore dei girini, somiglianti a quelli di un vero e proprio colon.

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Evidence for the Precipitating Activity of Insulin Antibodies

Today there is no doubt about the antigenicity of insulin. On the other hand, conflicting ideas exist about the antigenic character of the insulin molecule, since it has frequently been reported that insulin does not precipitate[1-3]. Above all, BERSON and YALOW[4,5] concluded that, in its reaction with antibodies in human antisera, insulin behaves as if it were univalent. This conclusion was drawn from studies on the sedimentation velocity of insulin-antibody complexes in the region of marked antibody excess, which showed that only a single antibody molecule combined with insulin. In contrast, however, precipitating antibodies to insulin have been obtained in the last 7 years in man, horse, guinea-pig, sheep and rabbit[6]. Finally, the study of ARQUILLA and FINN[7] presents evidence that the insulin molecule has more than one antigenic determinant site. Considering the frequently strong positive results obtained with the Boyden technique, neutralization tests, and complement fixation reactions[8], there is in principle no reason for assuming that insulin antibodies do not precipitate. Unfortunately, however, none of the reports about the precipitating activity of insulin antibodies affords a direct demonstration for such an activity, since it has been shown that many preparations of insulin recrystallized a number of times contain antigenic impurities showing no species specificity[9]. Our results, reported here in brief, give evidence for the precipitating activity of antibodies against insulin.

A group of 20 guinea-pigs was immunized with crystalline pig-insulin (free from glucagon; zinc content 0.29%), obtained from Farbwerke Hoechst. The animals received at weekly intervals a total of 10 injections of 1 mg insulin incorporated in complete Freund's adjuvant. The first 4 injections were given i.m., the next 4 doses s.c., the 9th i.c. and the 10th into the footpad. Shortly before and also 4, 8 and 12 h after the first 4 injections, each guinea-pig received a 6 ml i.p. injection consisting of 5% glucose. The animals were bled 10 days after the last injection. Ten guinea-pigs were likewise immunized with oxidized pig-insulin and 15 guinea-pigs with photooxidized pig-insulin. In the latter case, 6 of the 15 animals received with each injection 3 mg of the photooxidized preparation. The oxidative separation of pig-insulin into the sulphonates of the A- and B-chains was performed as described[10]. Test for the completeness of the separation was performed by means of paper electrophoresis, using 0.05 *M* veronal buffer (pH 7.4).

Photooxidized pig-insulin was prepared employing the photooxidation amino acid disruption method, using molecular oxygen in the presence of methylene blue as catalyst[11] as described elsewhere[12]. This procedure results in the preferential disruption of the imidazole ring, while tryptophane, tyrosine, methionine and cystine are essentially less involved and in decreasing degree. Photooxidation of insulin results in a complete loss of hormonal activity if all the imidazole rings are opened[13,14] even without changes in the tertiary structure of the insulin molecule[14]. The preparation obtained by us had a tyrosine content of 68%, while histidine was diminished to less than 5%, as determined photometrically[13].

The antisera produced against the various insulin preparations were investigated by the agar gel precipitation method, capillary precipitation and immunoelectrophoretic analysis.

Precipitating insulin antibodies could be found in antisera of animals immunized with either native or photo-

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