known[2,3] that some polyphenolases isolated from roots of higher plants catalyse the oxidation of some amino acids in the presence of phenolic substances giving rise to the red pigment. Some conditions of the red pigment origin were also studied in microorganisms[4-6]. White-rot fungi excrete numerous oxidases into the medium[7-9]. This is why we have used the 3% malt extract as the medium on which the fungi were grown as a source of raw enzymes for the formation of a red pigment in vitro. 1 ml $M/100$ of catechol, 1 ml $M/5$ of glycine and 1 ml $M/50$ 1-hydroxyproline were added to 1 ml of dialysed cultivation medium in 1 ml $M/10$ phosphate buffer, pH 5.9. This mixture was incubated at a temperature of 39 °C for 2 h. During this time it showed the formation of a red pigment, the absorption spectrum of which is shown in the Figure, curve 1b. The pigment was formed only in the presence of a cultivation medium in which the fungi *Trametes versicolor* and *Trametes gibbosa* were cultivated. It was, however, not formed in the case of the fungus *Fomes marginatus*.

From the results it follows that the fungi producing polyphenolases catalysing the secondary oxidation of amino acids in the presence of phenols, are able to form the red pigment during the wood decay in the way described above. Fungi which do not produce polyphenolases of the type mentioned, do not form the red pigment.

Zusammenfassung. Holzzerstörende Pilze, die ins Medium Polyphenolasen ausscheiden, bilden bei der Zersetzung des Holzes, welches mit 3% Peptonlösung aufgesogen wurde, ein rotes Pigment. Die Pigmentbildung ist durch die sekundäre Oxydation von Aminosäuren und die katalysierende Rolle der Polyphenolasen verursacht.

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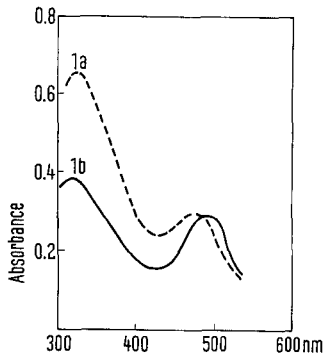

The absorption spectra of red colour solutions. Curve 1a: a red pigment isolated during wood decay by white-rot fungi. Curve 1b: a red pigment formed by the action of polyphenolase produced in the medium by white-rot fungi. Boiled enzymatic solution was used as blank.

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Postnatal Changes in Phosphatase and Non-Specific Esterase Activity in the Large Intestine of the Rat

During postnatal development of mammals there are changes in the histological structure of the gastrointestinal tract[1-5] and its enzyme pattern. This has been demonstrated histochemically[6-9] and biochemically[9-12]. The occurrence of enzymes in the large intestine during postnatal development has not received any attention at all, and only work on adult animals[13,14] has been performed. Since the large intestine may play a role in the absorption and metabolism of some substances[14], this has been studied during postnatal development of the rat in this paper.

Rats aged 1, 3, 7, 10, 15 and 20 days and adult animals were examined histochemically. Each age group contained 5 rats; sexes were represented about equally. Rats were decapitated 3 h after food deprivation. Excisions of the caecum and of the middle part of the oral and aboral half of the remainder of the large intestine were fixed in Baker's formol-calcium at 4 °C for 24 h, and alkaline and acid phosphatase were determined in paraffin sections together with AS- and α-esterase. ASD phosphate, ultrazol AS acetate, and α-naphthyl acetate were used as substrates; Fast red TR (Hoechst), hexatone basic fuchsin (Lachema) according to Barka, Fast blue RR (Hoechst) and Fast blue B (Lachema) were used as diazotates.

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Incubation times were 35 min for alkaline phosphatase, 60 min for acid phosphatase, 35 min for AS-esterase and 13 min for α-esterase.

The enzyme activities were located as described in the literature for the small intestine.

In general, alkaline phosphatase could be demonstrated in the brush border, apical and supranuclear Golgi zone of enterocytes, in the endothelium of some small vessels and capillaries, in eosinophils, neutrophils, some macrophages and reticular cells of lymphadenoid tissue. The activity of acid phosphatase is positive in the same locations, with the exception of the endothelium. Some mast cells contain acid but no alkaline phosphatase.

AS-esterase is positive as fine granules in the apical parts of enterocytes in the Golgi zone, less fine granules up to an intense diffuse reaction are found in the basal parts of the enterocytes in the gland crypts. Eosinophils, neutrophils, macrophages, fibrocytes and probably some mast cells are also positive. A slightly positive AS-esterase reaction was found also in smooth muscle cells of both muscular layers and the plexus myentericus. α-esterase shows a less pronounced reaction in the same locations and cannot be demonstrated in the mast cells.

The intensity of the reactions of the above enzymes is not the same along the whole large intestine of the same rat nor in the same sites in rats of different ages (Tables I and II).

In the enterocytes the phosphatases are pronounced in 1- to 7-day-old rats in the cilliary border and the Golgi zone of the caecum and the oral (proximal) middle part of the large intestine and appears as fine granules. The reaction is more intense for alkaline than for acid phosphatase. No enzyme reaction was found in the aboral part of the large intestine. During development the activity of the phosphatases decreases in the caecal enterocytes and the oral part of the large intestine. The positivity in the Golgi zone may disappear sooner than positivity in the brush border. In 10-day-old rats no acid phosphatase activity is found in the caecum; alkaline phosphatase activity disappears on day 20. In the endothelium, on the other hand, alkaline phosphatase activity increases from day 10 and is fairly pronounced in adult rats. No difference was found between rats aged 20 days and adult animals. In mono- and polynuclear leucocytes and fibrocytes the activity of both enzymes is present from day 1 and does not change; only the number of elements increases.

Table I

Age of rats (days)		Brush border			Supranuclear and apical zone			Endothelium		
		I	II	III	I	II	III	I	II	III
Alkaline phosphatase	1	+++	+++	–	++	++	–	–	–	–
	3	+++	+++	–	++	++	–	–	–	–
	7	+++	+++	–	++	++	–	–	–	–
	10	+	+++	–	–	+	–	±	±	±
	15	±	+	–	–	–	–	–	±	±
	20	–	–	–	–	–	–	+	+	+
	Adult	–	–	–	–	–	–	++	++	++
Acid phosphatase	1	+++	+++	–	+	+	–	–	–	–
	3	++	++	–	+	+	–	–	–	–
	7	±	++	–	+	+	–	–	–	–
	10	–	+	–	–	±	–	–	–	–
	15	–	–	–	–	–	–	–	–	–
	20	–	–	–	–	–	–	–	–	–
	Adult	–	–	–	–	–	–	–	–	–

AS-esterase and α-esterase activities are found in the enterocytes already on day 1, and activity increases from day 20. Activity is highest in adult rats, enterocytes of the aboral part of the large intestine also being positive in contrast to phosphatases. Slight positive reaction in poly- and mononuclear leucocytes and fibrocytes remains unchanged with age.

There are no data on the postnatal development of the large intestine. To our knowledge papers only deal with the comparative morphology of enzymes in the large intestine in various species and mainly deal with the small intestine. Thus the histochemical distribution of alkaline phosphatase in the large intestine differs in different species[13,14]. In the cat a strong positive reaction was found in the cilliary border along the whole large intestine, while in the rat this reaction is negative. These results agree with our findings in adult rats. In infant rats, on the other hand, phosphatase activity is still high.

From our investigation it may be speculated that during early postnatal development, and perhaps also prenatally, the large intestine participates in the absorption and metabolism of some substances. This will, of course, have to be proved.

Table II

Age of rats (days)		Brush border			Supranuclear and apical zone		
		I	II	III	I	II	III
α-esterase	1	–	–	–	±	±	±
	3	–	–	–	±	±	±
	7	–	–	–	±	±	±
	10	–	–	–	±	±	±
	15	–	–	–	±	±	±
	20	–	–	–	+	+	++
	Adult	–	–	–	++	+++/x	++/x
AS-esterase	1	–	–	–	+	+	+
	3	–	–	–	+	+	+
	7	–	–	–	+	+	+
	10	–	–	–	+	+	+
	15	–	–	–	+/x	+/x	+/x
	20	–	–	–	+/x	++/x	++/x
	Adult	–	–	–	++/x	++/x	++/x

I: caecum. II: middle part of the oral (proximal) half of the large intestine. III: middle part of the aboral half of the large intestine. Enzyme activity: + slight, ++ middle, +++ strong, – none, /x positive reaction also in the basal parts of the enterocytes in the gland crypts.

Zusammenfassung. Phosphatasen- und Esterasenaktivität in Enterozyten des Rattendickdarms ändert sich während der postnatalen Entwicklung. Alkalische und saure Phosphatase ist bei neugeborenen und jungen Ratten im oralen Dickdarmabschnitt stark positiv. Die Annahme der Beteiligung des Dickdarmes an Resorption und Metabolismus wird vorausgesetzt.

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