Toxohormone from Normal Tissues

Abstract. *The presence of toxohormone-like factor in autolysates of animal tissues was investigated. One could always isolate appreciable quantities of preparations having toxohormone activity. At the doses tested, no activity could be shown in preparations from nonautolyzed tissues. We suggest that autolysis phenomena may cause the presence of toxohormone in tumor-bearing organisms.*

The production of toxohormone in appreciable quantities has long been considered an exclusive and universal property of malignant cells (*1*). However, in 1961 Callao and Montoya (*2*) showed that toxohormone-like factors could be isolated from yeasts having impaired respiration, and Kampschmidt *et al.* (*3*) have since obtained preparations possessing high toxohormone activity by using different types of bacteria. Kampschmidt *et al.* claimed that toxohormone is not a product of tumorous tissues but is produced by the bacteria that contaminate these tumors; even if one ignores this claim, which has been disputed (*4*), it seems certain from the reports mentioned that the production of toxohormone-like products is not specifically by tumorous tissues.

Recently we have shown (*5*) that when ordinary baker's yeast is submitted to a process of autolysis under determined conditions one can obtain preparations having high toxohormone activity from the bacteria-free autolysates, whereas preparations obtained by use of nonautolyzed yeast possess little or no activity. These results have made us suspect that production of toxohormone in tumor-bearing organisms also may be due to autolytic processes occurring in the malignant cells or in the tissues invaded by them, so our experiments were designed to show whether the autolysis of normal animal tissues causes the production of toxohormone in appreciable quantities. We used tissues of skeletal muscle, lung, kidney, and liver of ox and rabbit.

The tissues were removed immediately after death and homogenized in a Waring blendor with double their weights of distilled water; the homogenates were then immediately incubated at 37°C under toluene, the duration varying with the type of experiment. When incubation ended, autolysis was stopped by the addition of 3 volumes of boiling ethanol, and each mixture was extracted at 80°C for 30 minutes, with constant stirring. Each mixture was left to cool and filtered; after pressing, the solids were extracted at 100°C for 1 hour with an equal weight of distilled water. The resulting extract, after filtration and concentration in a partial vacuum to 10 percent of its original volume, was precipitated with 2 volumes of ethanol. Finally, the precipitate so obtained was dried in a partial vacuum and extracted by the technique used to obtain crude toxohormone from tumorous tissue (*6*). In order to test bacterial contamination of the autolysates, samples were taken every 24 hours during the autolysis process, and the numbers of aerobic and anaerobic bacteria were determined by a normal plate-counting method on nutrient agar at 37°C.

In each instance a corresponding preparation was made by homogenizing nonautolyzed tissue directly with twice its weight of a mixture (3 : 1 by volume) of ethanol and water in order to avoid any initiation of the autolysis process during the homogenization, and by then applying the techniques described in the last paragraph.

Activities of the different preparations were tested by injecting suitable quantities into mice and then determining the catalase activity of the liver by the technique of Bonichsen *et al.* (*7*); it was expressed in terms of the monomolecular reaction-rate constant divided by the dry weight (grams) of the amount of the preparation (*8*) that was used in the analysis.

Table 1 shows the liver catalase activity in mice injected with preparations obtained from nonautolyzed tissues and from the same tissues after autolysis for 96 hours; each result is the average for five experiments. The preparations obtained by use of the autolysates significantly reduced liver catalase activities, whereas preparations obtained from the same tissues without autolysis did not cause significant reductions, despite the fact that they were injected at 40 mg per mouse versus 25 mg for the former. The quantity of toxohormone obtained from ox muscle, as well as the minimum dose that significantly reduces liver catalase activity when injected into mice, varies with the duration of autolysis (Table 2).

In any case, bacterial contamination

Table 1. Liver catalase activity in mice injected with toxohormone preparations (TH) obtained from normal animal tissues either fresh or submitted to a process of autolysis; it is the monomolecular reaction rate constant divided by the dry weight (grams) of the preparation that was used in the analysis.

TH prepared from:	Autolysis, duration (hr)	Mice (No.)	Injection per mouse (mg)	Liver catalase activity
Rabbit skeletal muscle	0	5	40	55.0 ± 4.05
	96	5	25	37.9 ± 2.94*
Rabbit lung	0	5	40	51.6 ± 3.60
	96	5	25	45.3 ± 2.80*
Rabbit kidney	0	5	40	64.2 ± 1.77
	96	5	25	41.8 ± 1.62*
Rabbit liver	0	5	40	65.9 ± 3.32
	96	5	25	32.7 ± 2.85*
Ox skeletal muscle	0	5	40	58.6 ± 3.01
	96	5	25	41.1 ± 2.00*
Ox lung	0	5	40	58.8 ± 2.30
	96	5	25	40.0 ± 3.13*
Ox kidney	0	5	40	60.3 ± 1.02
	96	5	25	32.4 ± 3.08*
		Controls		
		10		62.8 ± 2.85

* Significantly different from the blanks ($P < .01$).

Table 2. Yield (milligrams per 100 g of dry muscle at 105°C) of toxohormone from autolysates of ox muscle in relation to the duration of autolysis. Minimum active dose is the minimum quantity of toxohormone that significantly reduces liver catalase activity when injected into mice.

Autolysis, duration (days)	Yield (mg)	Minimum active dose (mg)
0	240	40
1	320	40
2	483	40
3	680	20
4	690	15
5	720	15
7	680	20
9	600	30
12	630	35

of autolysates was negligible: not more than 100 aerobic bacteria and 10 to 20 anaerobes were found per milliliter of autolysate during the process of autolysis. Thus we conclude that the autolysis of normal animal tissues, as of baker's yeast (*5*), leads to production of appreciable quantities of toxohormone, at least in the cases tested. In our opinion the phenomenon of autolysis may be the origin of the small quantity of toxohormone that can be isolated from all normal tissues (*8*). Likewise we suggest the possibility that the presence of toxohormone in tumor-bearing organisms is closely related to the processes of autolysis in the malignant cells or in the tissues invaded by them.

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Wrinkling of Molar Crowns: New Evidence

Abstract. *We submit that wrinkling of molar crowns in Primates is a phenomenon of genetically controlled activity of the inner enamel epithelium that is grossly evident soon after initial calcification of the tooth occurs. The amount and pattern of this wrinkling are characteristic of the species before they are evident in the enamel surface itself.*

In 1887 Schlosser (*1*) called attention to the occurrence of wrinkles in the enamel covering of the molars of fossil Hominoidea (namely *Dryopithecus*); he considered this feature to be of phylogenetic significance within the primate order. Even earlier, however, the arrangements of these enamel folds among the living Hominoidea were well known: in the gibbon this trait is the most attenuated, while in the orangutan it reaches its most profuse expression. The other anthropoid apes and man fall between gibbon and orangutan in this respect. The phylogenetic interpretation of this trait has been disputed in the literature: on the one hand, Adloff (*2*) dismissed wrinkling as a diagnostic taxonomic aid, since he considered this phenomenon to be simply a haphazard and inconstant accident of the calcification process; on the other hand, Korenhof (*3*) and Weidenreich (*4*), both of whom comprehensively reviewed both sides, felt that wrinkling in hominoids is at least as old as the Miocene and has phylogenetic and racial significance. Korenhof based his argument upon the fact that wrinkling appears on the surface of the dentino-enamel junction, which he regarded as a structure representing an earlier or "more original stage" of the tooth than does the enamel surface of the crown. The presence of wrinkling on the junction had been established as early as 1917 by Aichel (*5*); later by Schwarz (*6*). Weidenreich (*7*) put it this way: "As the dentine, regarded from the morphological viewpoint, is the most integral constituent of the tooth, its surface relief cannot be considered as a purely accidental feature with no morphological significance."

Since the problem of wrinkling is important not only in comparative primatology, hominoid evolution, and race differentiation, but also in dental embryology, we were moved to observe directly the status of the crown surface before, during, and after the calcification process. This we did by extracting the appropriate tooth buds from term fetuses of gorilla (*Gorilla gorilla gorilla*), orangutan (*Pongo pygmaeus*), and man, and by comparing them to the unworn crowns of the corresponding erupted molars of juvenile specimens of the three primate groups.

Figure 1 shows the mandibular right first permanent molars of gorilla, orangutan, and man in the unworn state. The characteristic profuse wrinkling of the crown surface of the orangutan contrasts with the relatively smooth surface of the human molar and with the rugged marked ridges, with the high cusps, on the gorilla molar. Figure 2 shows the mandibular right second primary molars (dm2) of the three species from the distal occlusal view; all three were removed from full-term fetuses. Only in the gorilla is the crown surface not entirely covered by calcified tissue; at this stage of development the enamel is immature and relatively shallow in depth. The crown surfaces were carefully dried before photography to eliminate highlights. The extensive wrinkling on the surface of the orangutan molar is quite obvious but less pronounced than in the completed tooth (Fig. 1). In the human molar the cusps are much steeper and the ridges descending from them are more strongly developed, but there are very few accessory ridges as in the orangutan. In the gorilla, sharp ridges occur on the slopes of the cusps, but at the bases of the cusps there is very little wrinkling.

The first permanent molars of the same full-term fetuses are shown in occlusal view in Fig. 3. In the gorilla three cusps have begun calcification: the mesiobuccal (Protoconid), mesiolingual (Metaconid), and distobuccal (Hypoconid). In the orangutan the same three cusps, plus the distolingual cusp (Entoconid), have commenced to calcify. In the human all five cusps are in various stages of calcification. In none of the molars, however, has coalescence of enamel between cusps occurred. The uncalcified portions of the crown show interesting differences. In the orangutan the surface is covered with minute ridges suggestive of the final crown form. In the gorilla there are fewer but heavier ridges located mainly on the steep slopes of the cusps. In the human the basin is broader and flatter and shows no wrinkling whatever.

It is apparent that wrinkling is not a phenomenon initiated in the enamel-formation process; it commences in the inner enamel epithelium. Presumably the wrinkling begins soon after initial calcification of one or more cusps in gorilla and orangutan. In man, wrinkling has not been observed in the molars even after all five cusps have calcified and coalesced; apparently any folding of the inner enamel epithelium that does take place occurs immediately in advance of the calcification process, and then only on the slopes of the cusps.

We agree with Weidenreich that the enamel folds and wrinkles found on primate molars do not result from fortuitous piling-up of enamel, although his reasons for asserting that these minute structures are integral parts of the tooth were based upon a commonly held notion of the significance of the dentino-enamel junction. Contrary to Schour's opinion (*8*) that the junction is the "blueprint pattern" for the final