infinity, since the limit approached by a power function as the exponent approaches infinity is an exponential when the function is plotted between fixed limits. Although the differences among the means were not large, the judgment of nonlinearity was clearly minimal for the intensity-time function with an exponent of 1.67. This outcome suggests that the psychophysical function for loudness is a power function with an exponent of 0.6, the reciprocal of 1.67. As shown in Fig. 1, the inverse of the logarithmic function—the exponential function—was judged to be among the most nonlinear of the functions examined.

On the basis of a weaker assumption, namely, that the observers' estimates merely reflect rank-order (judgments of "greater" or "less"), the median ordinal rank assigned to each stimulus was calculated and plotted as a triangle in Fig. 1. The numerical estimates by each observer were ranked, and the median rank was taken for each stimulus. Since the eight stimuli were presented twice to each observer, the rank-orderings went from "1" (least nonlinear) to "16" (most nonlinear). The rank-order measures accord with the geometric means in showing that the power function with an exponent of 1.67 resulted in the most linear increase of loudness with time. For various observers, the estimates of apparent nonlinearity were minimal for different exponents of the intensity-time growth functions. The minimum was at 1.11 for three observers, at 1.67 for seven observers, at 2.50 for five observers, and at two other values for three observers. The minimum was indeterminate for three observers. Thus the psychophysical functions for 15 of the 21 observers were power functions with exponents between 0.4 and 0.9 (the reciprocals of 2.50 and 1.11).

Although the foregoing results do not determine a precise value for the loudness exponent, they are consistent with the value 0.6 which has been recommended for engineering purposes by the International Standards Organization. The results also accord with those of experiments in which observers assigned numbers to represent loudness rather than the growth of loudness (*5*).

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Inactive Alkaline Phosphatase in Duodenum of Nursling Mouse: Immunological Evidence

Abstract. *A preparation of duodenal alkaline phosphatase from 11-day-old mice contains an enzymatically inactive material that cross reacts with antiserum prepared against one isozyme of 20-day alkaline phosphatase. After precipitation of this material from solution, the antiserum cannot precipitate the 20-day enzyme.*

Differentiation is often characterized at the chemical level by the appearance of or increase in enzyme activity that is proper to a type of cell or tissue under investigation. Although such an increase in activity has, in a few cases, been shown to be due to *de novo* synthesis (*1*), an alternative mechanism is the activation of a previously existing inactive form of the enzyme. In adult animals, enzymes such as phosphorylase (*2*) and tryptophan pyrrolase (*3*), which are subject to sudden metabolic stimulation, may be present in an inactive form. In developmental stages there is little evidence for similar phenomena, although grasshopper eggs are known to contain a protyrosinase that can be activated by heat (*4*) and fetal rat liver has an inactive *p*-hydroxyphenylpyruvate oxidase that may be activated by glutathione (*5*). However, no enzyme has been shown to be preceded in development by an immunologically similar precursor.

During postnatal development, the alkaline phosphatase of mouse duodenum exists in several forms that are characterized by differences in electrophoretic mobility, elution pattern from DEAE-cellulose (diethylaminoethyl) columns, and ratio of activity with phenylphosphate to that with β-glycerophosphate (*6*). A form of the enzyme with ratio of phenylphosphate to β-glycerophosphate of about 0.7 is found up to about 12 days of age. It then begins to be replaced by two new isozymes, one of which has a ratio above 6.0 and the other has a ratio of about 2.0 (*6*). The new isozymes reach their peak at 20 days. Since the administration of inhibitors of RNA and protein synthesis to young mice accelerate the appearance of the high-ratio activity, it has been suggested that the new forms are the result of conversion of precursor molecules of different catalytic properties (*7*). We have now investigated this possibility by an immunological method.

An antiserum was produced against high-ratio phosphatase (HR) that was prepared by passing a butanol extract of 20-day duodenum through a column of DEAE-cellulose (*6*). Such an antiserum can precipitate the greater part of added HR from solution (Table 1, lines 1–3). However, when the same amount of antiserum was mixed with an 11-day column eluate of low-ratio phosphatase (LR), a precipitate was observed. However, all but a negligible amount of the phosphatase activity remained in the supernatant (Table 1, lines 4–6).

All enzyme and antiserum preparations were centrifuged before combination in order to eliminate any possibility of nonspecific precipitation. No precipitate formed in the combination of LR with normal serum from the same rabbit. Therefore, the precipitation in the second case must have been caused by a serological reaction of something other than LR with the antiserum.

There are two possibilities: (i) the enzyme preparation contained a contaminating molecule that reacted with another antibody in the antiserum; or, (ii) the preparation contained an enzy-

Table 1. Phosphatase activity in supernatant and precipitate after combination with a constant amount of antiserum in the presence of 0.15*M* sodium chloride. Tubes were centrifuged after 30 to 36 hours at 4°C and the precipitates were dissociated with 0.2*M* carbonate buffer, *p*H 9.8, and brought up to the original volume. Enzyme activity was determined at *p*H 9.8 by measuring the released phenol (*9*). All activities are expressed as micrograms of phenol per milliliter of the enzyme-antiserum combination per 30 minutes. (The sum of the activity of the supernatant and precipitate is higher than the original activity because of an activating effect of serum components other than antibody on the enzyme.)

Enzyme	Initial activity	Substance added	Activity added	Activity supernatant	Activity precipitate
HR	633	Antiserum to HR		165	571
HR	633	Antiserum to HR		135	519
HR	633	Antiserum to HR		109	574
LR	710	Antiserum to HR		823	
LR	710	Antiserum to HR		783	21
LR	710	Antiserum to HR		783	3
LR supernatant	783	HR*	633	1432	40
LR supernatant	823	HR	633	1479	22
LR supernatant	783	Antiserum to LR		32	699
LR and HR	1343	Antiserum to HR		1268	271

* Concentrated HR was added so that change in the volume of the supernatant was negligible.

matically inactive molecule that could react with the antibody to the HR form. In order to determine which of these alternatives was correct, we tested the supernatant to see whether it still contained antibody to HR after reacting with LR material.

High-ratio phosphatase was mixed with the supernatant after centrifugation of the LR and antiserum combination. As shown in Table 1 (lines 7 and 8), the same concentration of HR was used as in the previous experiment in which HR had combined with the antiserum. After 36 hours at 4°C the tubes were centrifuged, and it was found that very little phosphatase had precipitated from the combination of HR with the LR and antibody supernatant. After reaction with the LR material, the antiserum to HR had apparently lost its ability to precipitate the HR enzyme.

It is well known that antigen-antibody complexes are soluble in the presence of excessive amounts of antigen (*8*). Although the activities of the HR and LR forms were adjusted to approximate equality in this experiment, there is a substantial difference in the protein content of the two isozyme preparations, so that the LR tubes contained approximately four times as much antigen protein as those that contained HR. The possibility that the excessive protein or some other factors in the LR experiments inhibits precipitation of an LR and antibody complex has, however, been ruled out by three findings: (i) An enzymatically inactive precipitate was seen when the LR form was combined with the antiserum, indicating that something could be precipitated from this mixture. (ii) When antiserum against the LR form was added to the mixture of LR and antiserum to HR, most of the LR was precipitated (Table 1, line 9), showing that phosphatase can be precipitated from this mixture. (iii) When the same amounts of HR and LR as were used in the previous experiments were combined and then treated with the same quantity of antiserum to HR, a precipitate formed that had phosphatase activity (Table 1, line 10). Since some of the HR antibody would be removed by an inactive cross-reactive component in the LR enzyme preparation, it could be anticipated that less active enzyme would be precipitated from the HR and LR mixture than from the solution that contained only HR, and this is what happened (compare line 10 with lines 1–3 in Table 1). Although these experiments do not rule out the possibility that an excess of antigen exerts some influence on the amount of precipitation formed, the observation that substantial precipitation of phosphatase can occur from this mixture shows that the failure of precipitation of HR with antiserum to HR absorbed with LR (lines 7 and 8 in Table 1) is not the result of an excess of antigen.

We therefore conclude that a molecule that is enzymatically inactive but immunologically similar to HR exists as early as 11 days. The fact that this material is present in the phosphatase peaks eluted from DEAE-cellulose columns indicates that it is not just a small molecule or hapten, but a protein that has the same elution characteristics as the active phosphatase. The possibility that the material is an unstable phosphatase inactivated during purification seems to be ruled out by the fact that 11-day extracts lose very little activity on passage through DEAE-cellulose, and the activity eluted has the same low ratio as the homogenate from which it was prepared. The presence of this inactive form suggests that the appearance of HR beginning at 12 days may be partially caused by an activation of this inactive form.

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X-ray Detection by the Olfactory System: Ozone as a Masking Odorant

Abstract. *The technique of masking was used to test the hypothesis that x-ray detection is mediated by an odorant produced in irradiated air. Rats conditioned to cease licking during exposure to x-ray (conditioned suppression) did not display this conditioned response in the presence of ozone and strong volatile oxidants.*

A role has been established for the olfactory system in the detection of low doses of x-ray by rats. Odorants, the olfactory membrane, nerve, and bulb have been considered (*1*) among possible mediators of this effect. The discovery that alcohol on the nasal mucosa blocks x-ray activation of olfactory neurons (*2*) led to the hypothesis that detection is mediated by the production of odorants within the nasal passage or by direct action of radiation on the olfactory sensory cell.

Because x-ray is known to produce active molecules such as ozone and oxides of nitrogen (*3*), we first under-