

Reiko Yanai, Hiroshi Nagasawa, and Kazuo Kuretani\*<sup>1</sup>: Effect  
of Terephthalic Acid on the Bound Dye Level in the  
Liver of Rat fed *p*-Dimethylaminoazobenzene.

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It has been reported in the authors' previous experiment that terephthalic acid (TPA) supplemented to the diet containing *p*-dimethylaminoazobenzene (DAB) protected the activities of DAB metabolizing enzymes in the rat liver against their remarkable decreases by DAB feeding.<sup>1)</sup> Further, the protein bound dye level in the liver after 4 weeks of TPA feeding was found to be lowered to about 64% of the control.<sup>1)</sup>

Miller and Miller<sup>2)</sup> demonstrated that the protein bound dye level in the liver of the rat fed high riboflavin diet was lower and the maximum time, when the protein bound dye level reached maximum, was later than those of the control, and that the diet also delayed the incidence of hepatomas by 6 months.

Hence, it may be advisable to investigate the effect of TPA on the formation of bound dye with the possibility in view that TPA may play some role in preventing DAB carcinogenesis in the rat liver. The present experiment was carried out in order to reveal whether TPA feeding delayed the maximum time in the bound dye level and/or it lowered the maximum value itself, and whether TPA facilitated the degradation of bound dye once formed.

### Experimental

**Animal**—The animals used were male rats of the Wistar strain weighing 200~250 g. The feeding conditions were essentially similar to those in the previous report.<sup>1)</sup>

**The Effect of TPA on the Change of Bound Dye Level**—The animals were divided into two groups. One group (Control) was fed 0.06% DAB added to the basal diet, and the other (TPA group) was offered the further addition of 2% TPA. The composition of the basal diet was the same as in the previous experiment.<sup>1)</sup> These diets and water were supplied *ad libitum*. Six rats in each group were sacrificed every other week during the experimental feeding for 14 weeks, and the protein bound aminoazo dye in the liver was assayed.

**The Effect of TPA on the Degradation Rate of Bound Dye**—After the DAB feeding for 4 weeks, six out of 42 rats were sacrificed and the bound dye level in the liver was determined. The rest of the animals were divided into the control and TPA groups. The former was offered the basal diet only and the latter the diet supplemented with 2% TPA to the basal one. Six rats each were sacrificed at 2, 3, and 6 days after the end of DAB feeding for the bound dye assay.

**Assay of Protein Bound Aminoazo Dye**—The assay procedure of protein bound aminoazo dye was similar to that by Miller and Miller.<sup>2)</sup> The value was expressed as  $E(\log_{10} I_0/I)_{520}$  m $\mu$  per 100 mg. dry weight of liver, from which the value of normal rat liver was subtracted.

### Results

#### The Effect of TPA on the Change of Bound Dye Level

The change of bound dye level in each group is shown in Fig. 1. The maximum times were observed at the 4th and 8th weeks in the control and TPA groups respectively, and the maximum value in the latter was about 72% of the former. The difference was statistically significant ( $0.299 \pm 0.011$  *v.s.*  $0.417 \pm 0.026$  E/100 mg. dry weight) ( $P < 0.01$ ).

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1) R. Yanai, K. Kuretani: This Bulletin, in press.

2) E. C. Miller, J. A. Miller: Cancer Res., 7, 468 (1947).

The bound dye levels till the 8th week were significantly lower in TPA group than in the control ( $P < 0.05$  or  $0.01$ ). The change of bound dye in the control obtained in the present experiment had the similar tendency to the result by Miller and Miller.<sup>2)</sup>

The decreases in the body weight of both groups were about 10% of the initial weights irrespective of feeding periods. The increases in liver weights were not observed both in the TPA and in the control groups till the 8th week, but after the 10th week, the liver weight in the control began to increase.

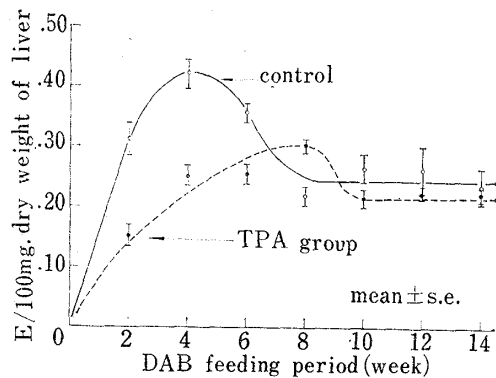


Fig. 1. Effect of TPA on the Bound Dye Level

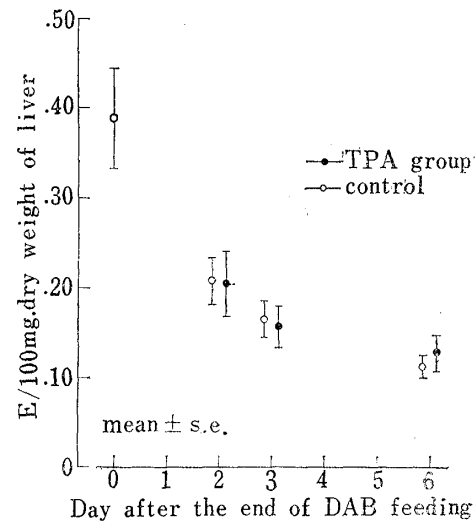


Fig. 2. Effect of TPA on the Degradation Rate of Bound Dye

### The Effect of TPA on the Degradation Rate of Bound Dye

The degradation rate of bound dye in each group is presented in Fig. 2.

The bound dye levels on 2, 3, and 6 days after the end of DAB feeding in both groups were about 47, 33, and 22% of the value on the last day of DAB feeding, respectively, and no difference in the degradation rate was found between the groups. This result may substantiate that TPA had no enhancing effect on the destruction of bound dye once formed under the present experimental condition.

### Discussion

The present experiment has demonstrated that the maximum time of bound dye was delayed about 4 weeks and the maximum value itself was about 72% in the TPA group when compared with those of the control.

Miller and Miller<sup>2)</sup> postulated that the formation of the dye protein compounds might be one of the initial phases of the carcinogenic process induced by DAB, and that the amount of bound dye would be the resultant of two reactions, the rates of the bound dye formation and of destruction, suggesting that similar levels of bound dye could result from combinations of different rates of formation and destruction. Further they advocated that this possibility should be taken into account in comparing the bound dye levels in the livers.

As the present experiment found no effect of TPA on the destruction of bound dye once formed, TPA is considered to play some role in disturbing the bound dye formation. The authors' previous result that TPA restored the activities of DAB metabolizing enzymes in rat liver<sup>1)</sup> may account for, in part, the less formation of bound dye by TPA in the present experiment, although TPA may interfere with binding of DAB to liver protein by some other mechanism. In any case, it is possible that TPA may play some role in preventing or delaying the hepatic carcinogenesis by DAB.