Table 1. Polonium content of mainstream smoke (from cigarettes of one brand) after various types of filtering. "Material" is material trapped by the membrane filter. Abbreviations: Rep exp, replicate experiments; cha, charcoal; cel, cellulose; res, resin.

Filter, type	Cigarette per sample (No.)	Rep exp (No.)	Material (mg)	Mainstream content per cigarette (pc)		Po:smoke (pc:mg)
				Average	S.E.	
None	20	2	0.0174	0.0156	0.0000	0.896
Cha + cel	20	2	.0052	.0078	.0000	1.500
Res + cel	20	2				
Res + cel	10	5	.0086	.0012	.0004	0.140
Res + cel	8	5				
			Content in resin			
	8	5		0.0136	0.0007	

ion-exchange resin; the resin from some of these cigarettes was analyzed for ^{210}Po content to confirm our opinion that the polonium removed was on the resin. In all instances the material deposited from the mainstream smoke on the membrane filters was analyzed for ^{210}Po by a reported method (*3*). Of the 11 packs of cigarettes used by us, ten came from the same carton so that possible variation in polonium content among cartons of the same brand would be minimized—a step justified by the lower polonium content in the mainstream smoke of this batch of cigarettes. Our other analyses of this brand of cigarettes, without filters, had shown higher contents (0.0263 pc per cigarette, in the mainstream) similar to those found by other authors. The resin used was a 20- to 50-mesh mixture (1:1) of cation- and anion-exchange resin (*4*) intended for water-demineralizer cartridges. The resin was removed from the cartridges and used as is.

All results (Table 1) have been corrected for reagent blank values, background, and recovery. They indicate that the normal filter on this brand of cigarettes removes 50 percent of the polonium in mainstream smoke, and that resin substituted for the charcoal removes 92 percent or more.

The polonium content of mainstream smoke was so low that indirect methods were used to confirm these results. From one pack of cigarettes, five with the filter intact were smoked through each of two tared membrane filters. The same was done with five cigarettes in which the charcoal was replaced with resin. The weight of residue collected on the membrane filter indicated that it collected on average 3.4 mg more material from the mainstream of each of the resin cigarettes than of each of the intact cigarettes. In part this finding reflected the fact that more of the resin cigarettes was smoked (the length of their butts averaged 40.5 mm against 44.2 mm for the intact cigarettes), but it may be that charcoal removes more organic material from mainstream smoke than does the resin.

To determine whether the lowered content of polonium in the mainstream smoke was due to removal by the resin, the resin from the five replicates listed in the last row of Table 1 was analyzed. The sum of this average value and the average of picocuries per cigarette, in mainstream smoke from the resin-filtered cigarettes, should equal the average number of picocuries per cigarette in mainstream smoke from unfiltered cigarettes. Failure of a *t*-test of this hypothesis to reject at the 95-percent level implied that we had accounted for all the polonium in mainstream smoke. A similar test of the hypothesis that the second and third values in column 5 of Table 1 are equal was rejected at the 99-percent level.

We conclude that exposure of smokers' lungs to alpha activity is markedly reduced by incorporation of an ion-exchange resin in cigarette filters. This conclusion is based on the reasonable assumption that mainstream polonium occurs in a similar form in all domestic cigarettes. A crude cost analysis indicates that incorporation of resin would be a relatively inexpensive control measure: the 0.12 g of resin used per cigarette would cost about 0.5 cent per pack. This type of radiation control could be applied while further radiobiologic experiments are being designed to test the significance of the ^{210}Po content of mainstream smoke.

ERICH W. BRETTHAUER
STUART C. BLACK
Southwestern Radiological Health Laboratory, U.S. Public Health Service, P.O. Box 684, Las Vegas, Nevada

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20 March 1967

Failure of Cycloheximide To Induce Tyrosine Transaminase in the Anesthetized Rat

Abstract. *It was recently reported that cycloheximide, an inhibitor of protein synthesis, induces tyrosine transaminase in the liver of adrenalectomized rats. We have been unable to confirm this effect in the anesthetized animal and our data show that cycloheximide inhibits the induction caused by hydrocortisone in adrenalectomized rats or by stress in intact rats.*

The activity of tyrosine transaminase (L-tyrosine: 2-oxoglutarate aminotransferase, EC No. 2.6.1.5) is markedly stimulated in rat liver by hydrocortisone (*1*), this stimulation being due to *de novo* synthesis of protein (*2*). It was recently reported by Fiala and Fiala (*3*) that cycloheximide, an inhibitor of protein synthesis (*4*), induces tyrosine transaminase to a value 250 to 300 percent above basal level in 4 hours. The effect was observed in the livers of both intact and adrenalectomized rats, and the increase was blocked in the adrenalectomized animal by puromycin. Moreover, Fiala and Fiala's data indicate that the antibiotic enhances the induction of tyrosine transaminase by hydrocortisone.

We were particularly interested in this report in view of recent developments concerned with regulatory processes affecting the rate of synthesis of tyrosine transaminase in rat liver. Kenney (*5*) has presented evidence supporting the view that this enzyme is normally repressed at the translation step of its biosynthesis by a repressor with a very rapid turnover and that induction by hydrocortisone could

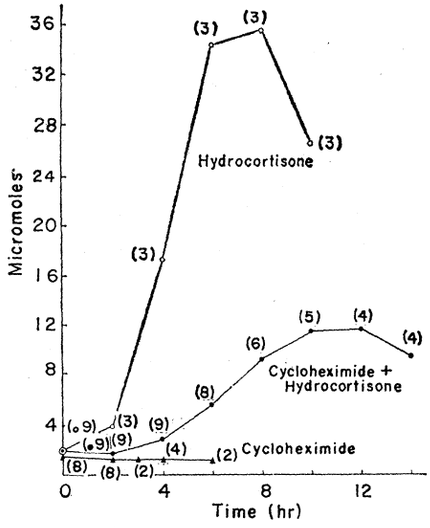

Fig. 1. Effects of cycloheximide on the basal level of tyrosine transaminase and on its induction by hydrocortisone in adrenalectomized rats. The drugs were administered just after the zero-time biopsy. The numbers in parentheses indicate the number of animals actually carried through the indicated length of time and from which biopsy samples were obtained at all previous points. The activity is expressed as micromoles of *p*-OH-phenylpyruvic acid formed per hour, per 10 mg of protein, at 25°C.

be partially due to the release of repressor restraint. According to this model, the paradoxical induction of tyrosine transaminase by cycloheximide might be explained in terms of a differential inhibition of repressor formation versus enzyme synthesis, favoring the latter. The net effect would be an early transient increase in enzyme amount such as reported by Fiala and Fiala (*3*).

In our studies we have used the spectrophotometric method of Lin *et al.* (*6*) for the assay of tyrosine transaminase. Male, intact or bilaterally adrenalectomized rats of the Sprague-Dawley strain, weighing between 165 and 220 g, were supplied by the Canadian Breeding Laboratories, St. Constant, Quebec. Cycloheximide (Actidione, Nutritional Biochemicals) was administered intraperitoneally at a level of 1 mg/kg of body weight. Hydrocortisone sodium succinate (*7*) was given intraperitoneally at a dosage of 30 mg/kg of body weight. For protein determinations the method of Lowry *et al.* (*8*) was used. In our preliminary experiments the response of tyrosine transaminase to cycloheximide in the liver of adrenalectomized rats was as indicated in Table 1. Each experiment represents one group of animals as shipped by the supplier. The group was divided into saline control and cycloheximide-treated animals which were killed simultaneously. In only one experiment did the activity rise to a significant extent (experiment No. 3), while in the other two experiments it did not change significantly. It must be noted that all animals in some cycloheximide-treated groups succumbed 2 to 3 hours after treatment. The results suggest that, at best, the increase of tyrosine transaminase in response to cycloheximide was erratic and limited.

In recent studies on the regulation of tyrosine transaminase, we employed a serial biopsy technique, details of which are given elsewhere (*9*). In this technique each animal serves as its own control. It consists essentially of removing small liver samples (30 to 40 mg) through a midline incision in the abdominal wall of rats kept under pentobarbital anesthesia. A zero-time biopsy is taken before the administration of the substance under study. Administration of hydrocortisone to such anesthetized adrenalectomized rats results in large increases in tyrosine transaminase activity, with a peak at 6 to 7 hours and a return to basal levels in about 12 hours after administration of the glucocorticoid. In control animals the activity, after a slight initial reduction, remains practically constant.

We have used this technique, and Fig. 1 summarizes our experiments on the effect of cycloheximide on the basal level of the enzyme and on its induction by hydrocortisone in the liver of adrenalectomized rats. After a slight initial reduction the enzyme level remains practically unchanged for up to 6 hours after the injection of cycloheximide. No animal survived beyond this time. Cycloheximide given simultaneously with hydrocortisone inhibits the induction caused by hydrocortisone alone. The difference between the kinetics of the uninhibited and cycloheximide-inhibited induction by hydrocortisone is evident. Cycloheximide delays the onset of enzyme accumulation, and the latter continues for 3 to 4 hours after the induction cycle in the animals that are given hydrocortisone alone has entered the inactivation phase. The effect results in almost peak-level enzyme activity even after 14 hours from the start of the experiment. In these experiments, therefore, cycloheximide acted as an inhibitor of the hormonal induction and not as an inducer of tyrosine transaminase, and in its presence the kinetics of limited induction is similar to the kinetics established in the presence of puromycin (*10*).

Table 1 Effect of cycloheximide on liver tyrosine transaminase of male adrenalectomized rats. The animals were killed 4 hours after the administration of the antibiotic. Numbers in parentheses indicate number of animals.

Experiment No.	Liver tyrosine transaminase activity (micromoles of *p*-OH-phenylpyruvic acid per hour, per 10 mg of protein, at 25°C)	
	Saline control	Cycloheximide (1 mg/kg)
1	2.73 (4)	2.26 (6)
2	1.72 (4)	2.05 (3)
3	1.83 (4)	3.89 (6)

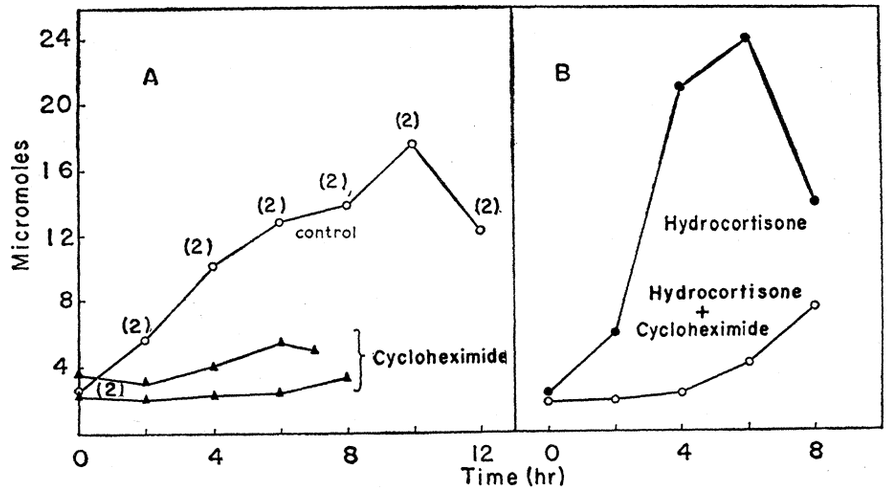

Fig. 2. Effect of cycloheximide in intact rats on the induction of tyrosine transaminase caused by anesthetic and surgical stress (A) and by hydrocortisone (B). All curves except the control represent single animals. Other details are as in Fig. 1.

In the course of our work we observed that the enzyme is induced in intact rats under the conditions of anesthesia and surgery without the administration of the glucocorticoid, presumably under the influence of a stress-mediated release of corticosterone. A five- to eightfold induction can thus be effected. Figure 2A illustrates the action of cycloheximide in such animals. The stress-mediated induction is again inhibited by the antibiotic. Without inclusion of the right controls the small elevations in tyrosine transaminase levels noted in the cycloheximide-treated animals would resemble a very modest enzyme "induction." In fact, they represent residual activities of the stress-mediated induction which is largely inhibited by the antibiotic.

Figure 2B illustrates the effect of cycloheximide on the induction of tyrosine transaminase by hydrocortisone in intact anesthetized rats. In agreement with Rosen *et al.* (*11*), we found that the response of the enzyme to hydrocortisone in these animals is lower than that in adrenalectomized animals. The kinetics of the induction and its inhibition by cycloheximide are quite similar to those established in the adrenalectomized rats (Fig. 1).

Our experiments demonstrate clearly that cycloheximide is not an inducer of tyrosine transaminase in the anesthetized rat and, rather than enchancing (*3*) the action of hydrocortisone, cycloheximide inhibits the inductions caused by the glucocorticoid in both intact and adrenalectomized rats as well as the stress-mediated induction in the intact animals. The significance of the small, erratic elevation of tyrosine transaminase noted in some animals (Table 1) is unclear. Because of the erratic nature of the response in these animals, further speculation on the possibility of differential inhibition of repressor formation referred to in the beginning of our report is not justified at this stage. It must be noted that in the report by Fiala and Fiala (*3*) elevations of the enzyme in cycloheximide-treated adrenalectomized rats are tabulated for only three animals against one control. Inclusion of more animals in this type of experiment might have resulted in eliminating a large part of the discrepancy between their results and ours. We have observed that large variations in basal levels of tyrosine transaminase do occur in adrenalectomized rats.

In conclusion, the speculation (*3*) that the reported elevations of tyrosine transaminase may represent a "pseudohormonal" induction in which hydrocortisone is replaced by cycloheximide for the induction of the enzyme is not consistent with our experimental data gathered by the use of the serial biopsy technique.

C. MAVRIDES
E. A. LANE
Department of Biochemistry, Faculty of Medicine, University of Ottawa, Ottawa, Ontario

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Calcium-Selective Electrode with Liquid Ion Exchanger

Abstract. *A simple calcium-selective electrode has been developed which is capable of measuring calcium ion activity in the presence of many common interfering ions. The electrode utilizes a liquid ion exchanger membrane containing the calcium salt of a disubstituted phosphoric acid.*

At the present time there are no ion-sensitive electrodes which have a sufficiently high selectivity for calcium ion in the presence of sodium and other monovalent cations to permit their use in the analysis of biological fluids for calcium ion activity. Typical biological samples have monovalent cation ion concentrations on the order of 100 times the concentration of uncomplexed calcium ion. This report describes a liquid ion exchange electrode capable of measuring free calcium ion activity in the presence of a 1000-fold excess of sodium or potassium ions.

The electrode was constructed from a glass tube, 1 cm in diameter, sealed at the lower end with cellulose dialysis tubing. The tube is filled to a depth of approximately 2 cm with a liquid ion exchange solution, 0.1*M* in the calcium salt of didecylphosphoric acid dissolved in di-*n*-octylphenyl phosphonate. Electrical contact is made to the organic phase via a narrow glass tube containing a 0.1*M* $CaCl_2$ aqueous 2 percent agar gel and a silver–silver chloride electrode. Potential measurements were made with a Corning model 12 *p*H meter.

The water immiscible organic phase in the tube forms a liquid ion exchange "membrane" whose ion exchange properties for cations are similar, in mechanism, to the liquid ion exchanger concentration cells reported by Sollner (*1*) and Bonner (*2*). To the extent that calcium is the only cation present in the sample which can participate in ion exchange with the organic calcium salt, then charge transport between the sample and the internal agar phase involves net movement of calcium ions only; that is, the transport number of calcium ions across the organic phase is unity. Under these conditions the equilibrium potential across the membrane is given by the Nernst equation:

$$E_{\text{memb.}} = \text{constant} + \frac{RT}{2F} \log A_{Ca^{++}} \quad (1)$$

where $A_{Ca^{++}}$ is the "ionic activity" of calcium ion in the aqueous sample phase. The value of the constant term depends on the particular choice of reference electrode and the activity of calcium ion in the internal agar phase.

It was first necessary to establish that the calcium electrode behaved in an ideal Nernst manner under conditions where no assumptions regarding ionic activity coefficients or liquid junction potentials were necessary. Table 1 shows data obtained with the cell

Ca electrode | $CaCl_2$ (*M*) | AgCl | Ag

Provided that the calcium electrode functions as a Nernst electrode for calcium ions the cell potential should be given by

$$E_{\text{cell}} = \text{constant} + \frac{3RT}{2F} \log M\gamma_{\pm} \quad (2)$$

where $\gamma_{\pm}$ is the mean ionic molal activity coefficient of calcium chloride and M is the molal concentration. The value of the constant depends on the activity of both calcium and chloride ions in the internal agar gel. Over a 4-decade concentration range from 10^{-4} to 1*M* an E_{cell} versus log $M\gamma_{\pm}$