properties similar to rapidly dividing normal cells such as intestinal mucosa, nor are they similar to those of relatively non-adhesive erythrocytes, lymphocytes or bone marrow cells. Ambrose and Easty[3] obtained evidence for slight selectivity using whole venom (Russell's viper) in comparative investigations of tumour and normal cells in culture.

Preliminary *in vivo* investigations using this fraction have been quite encouraging, and several animals have been cured of Yoshida ascites tumours; but *in vivo* effects involve problems connected with blood supply to the tumour, rate of elimination of the agent, etc. Whatever the final success obtained with this fraction *in vivo*, the experiments described here on lytic action indicate that the necessary specificity of a structure is present on the tumour cell surface in this case. It may be possible to exploit this by the use of biological macromolecules containing the required properties in their amino-acid, sugar or nucleotide sequence as carcinolytic agents.

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[1] Wieme, R. G., and Rabaeye, M., *Naturwiss.*, **44**, 112 (1957).
[2] Easty, D. M., Ledoux, L., and Ambrose, E. J., *Biochim. Biophys. Acta*, **20**, 528 (1956).
[3] Ambrose, E. J., and Easty, D. M., *Brit. Emp. Cancer Camp. Rep.*, 39–58 (1961).

Reversal of Selective Toxicity of (−)-α-Lipoic Acid by Thiamine in Thiamine-deficient Rats

INTRAPERITONEAL injection of (±)-α-lipoic acid has been reported to be toxic to thiamine-deficient rats[1]. Development of the toxic effect was prevented by simultaneous injection of 100 μg of thiamine hydrochloride. It was also noticed that (±)-α-lipoic acid, unlike neopyrithiamine, had no effect on thiamine excretion. The toxicity of (±)-α-lipoic acid to thiamine-deficient rats therefore could not be related to the depletion of the thiamine reserves present in the organs of thiamine-deficient animals. Obviously, the results obtained from the use of the racemic form of lipoic acid could not reveal whether both or one of the isomers had been responsible for the toxic effects in the deficient animals. In order to clarify the relative toxicity of the isomers of lipoic acid in thiamine-deficient rats, the experiments were repeated with the natural and unnatural isomers of α-lipoic acid. The preparation of pure (+)-α-lipoic acid was accomplished from (+)-6,8-dichloro-octanoic acid (−)-ephedrine salt[2]. However, the (−)-α-lipoic acid was found to be contaminated with 15–20 per cent of the dextro-rotatory enantiomorph[2]. Therefore, the pure (−)-α-lipoic acid was synthesized from the purified (−)-6,8-dichlorooctanoic acid (+)-ephedrine salt. This was obtained after converting a sample of (−)-6,8-dichloro-octanoic acid (−)-ephedrine salt into the (+)-ephedrine salt. (The (−)-6,8-dichlorooctanoic acid (−)-ephedrine salt was generously supplied by Dr. W. J. Wayne of Du Pont.)

In the experiments with the various forms of lipoic acid, 20 mg/kg of α-lipoic acid was administered intraperitoneally to thiamine-deficient Sprague–Dawley rats of 100–150 g average weight. When the animals developed the 'acute stage' of polyneuritis[3], they were divided into subgroups as shown in Table 1. After the daily collection of urine, the animals were fed in the morning and the food was withdrawn. When the urinary thiamine values fell below detection, some of the animals in each group received 250 μg of thiamine HCl intraperitoneally, followed by i.p. injection of lipoic acid or neopyrithiamine. The urine samples were 24-h collections. They were collected for three days and were daily analysed for thiamine excretion. The values in Table 1 represent the total thiamine excreted in three days. Earlier it was established[1] that the 20 mg/kg (±)-α-lipoic acid corresponded to 1/4 of the LD_{50} dose. Since intraperitoneal injection of isotonic saline was not lethal to the thiamine-deficient animals, it is apparent that the mechanical effect of injection *per se* is of no consequence. Again it is obvious from Table 1 that the simultaneous injection of thiamine and lipoic acid prevented death, which ordinarily occurred when lipoic acid was administered alone to the deficient rats. Interestingly, (−)-α-lipoic acid seemed to be extremely toxic to thiamine-deficient rats whereas its injection into thiamine-sufficient animals had not produced any undue physiological effects. As a further confirmation of earlier findings[1], it has been demonstrated that a single intraperitoneal injection of 250 μg of thiamine hydrochloride followed by the injection of any of the lipoic acids does not lead to increased urinary thiamine excretion during the succeeding 72 h. The results show that neither the racemate nor either of the isomers of α-lipoic acid had any effect on thiamine mobilization while intraperitoneally administered neopyrithiamine (5 mg/kg) produced significant increase in thiamine excretion. No significant difference between the amounts of thiamine excretion before and after lipoic acid treatment could be observed in the experiments with thiamine-sufficient rats. At present the marked toxicity of (−)-α-lipoic acid is interpreted as an interaction between the thiaminethiol of the thiamine and (−)-α-lipoic acid, yielding a toxic intermediate in the brain possibly through a heteromeric interaction between thiamine and sulphydryl groups as suggested by Banhidi[4]. Such interaction could yield a toxic intermediate which would not only tie up thiamine reserves in the brain but also could cause the displacement of the natural isomer of α-lipoic acid from the sites of enzymatic decarboxylation of α-keto acids. The search for existence of such an intermediate is proposed to be undertaken with the aid of labelled isomers of α-lipoic acid.

Table 1. TOXICITY OF (−)-α-LIPOIC ACID AND ITS EFFECT ON URINARY THIAMINE EXCRETION IN THIAMINE-DEFICIENT RATS

Compound	Mortality of deficient rats (%)	Urinary thiamine* in 3 days μg	%
(±)-α-Lipoic acid	75 (16)	—	—
(±)-α-Lipoic acid + Thiamine (250 μg)	0 (10)	69	28
(+)-α-Lipoic acid	40 (16)	—	—
(+)-α-Lipoic acid + Thiamine (250 μg)	10 (10)	64	26
(−)-α-Lipoic acid	80 (16)	—	—
(−)-α-Lipoic acid + Thiamine (250 μg)	20 (10)	70	28
Neopyrithiamine	27 (10)	—	—
Neopyrithiamine + Thiamine (250 μg)	0 (6)	150	60
Isotonic saline + Thiamine (250 μg)	0 (6)	60	24

The animals in the various groups received i.p. injection of 20 mg/kg of the different lipoic acids or 5 mg/kg of neopyrithiamine. *In some groups when the urinary thiamine excretion fell below detection, the animals received 250 μg of thiamine hydrochloride followed by injection of lipoic acids, or neopyrithiamine or saline. No. of animals is given in brackets.

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[1] Gal, E. M., and Razevska, D. E., *Arch. Biochem. Biophys.*, **89**, 253 (1960).
[2] Acker, D. S., and Wayne, W. J., *J. Amer. Chem. Soc.*, **79**, 6483 (1957).
[3] Kline, O. L., Tolle, C. D., and Nelson, E. M., *J. Assoc. Agric. Chemists*, **24** 147 (1938).
[4] Banhidi, Z. G., *Acta Physiol. Scand.*, **50**, suppl. 174 (1960).