an important role in the transport of anesthetic gases in the blood.

The literature concerned with the solubility of different anesthetic gases in tissues and blood of different species under uniform conditions using a single end point for the definition of "anesthesia" is extremely limited. Yet most theories of anesthesia, past and present, are necessarily based upon the composite values of different species and different degrees of anesthesia. The data presented here seem to indicate that for a single agent, xenon, one cannot extrapolate, from one species to another, even such a basic value as blood-gas concentration. The studies on the cyclopropane–serum albumin interaction indicate that protein–gas associations are complex systems and not as easily explained as might be hoped.

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REFERENCES

1. R. M. Featherstone, C. A. Muehlbaecher, F. L. DeBon and J. A. Forsaith, *Anesthesiology* **22**, 977 (1961).
2. C. A. Muehlbaecher, F. L. DeBon and R. M. Featherstone, *Intern. Anesthesiol. Clinics* **1**, 937 (1963).
3. R. M. Featherstone and C. A. Muehlbaecher, *Pharmacol. Rev.* **15**, 97 (1963).
4. C. Tanford, J. Buzzell, D. Rands and S. Swanson, *J. Am. Chem. Soc.* **77**, 6421 (1955).
5. W. Leonard, K. Vijai and J. Foster, *J. Biol. Chem* **238**, 1984 (1963).
6. A. Wishnia, *Proc. Natl. Acad. Sci. U.S.* **48**, 2200 (1962).
7. J. Adriani, "The Chemistry of Anesthesia," p. 402. Thomas, Springfield, Illinois, 1959.

Enhancement of 5-Iododeoxyuridine Incorporation into DNA of Cat Tissues *in Vivo* by Inhibition of Uridine-Deoxyuridine Phosphorylase

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SUMMARY

When ^{131}I-iododeoxyuridine (IUdR) (31 μmoles/kg) was given to cats, the *in vivo* incorporation into DNA of bone marrow, intestine, and spleen was increased 3- to 7-fold by deoxyglucosylthymine (TdG) given at a ratio IUdR:TdG = 1:2, and 5- to 14-fold at a ratio 1:5.

Contrary to the extensive incorporation of IUdR[1] into the DNA of bacteria, phages, and cells in tissue culture, extremely low incorporation is seen in living animals (1–5). This is due to the very rapid degradation of 5-halogenated deoxyuridines in the animal body (1, 2, 6), limiting the therapeutic effectiveness of these compounds. Despite repeated attempts to inhibit this process (2, 4, 7–9), no increase in incorporation produced in this way has been reported as yet. The following results show that it is really possible to improve IUdR incorporation into the DNA of animal tis-

[1] Abbreviations: DNA = deoxyribonucleic acid; IUdR = 5-iododeoxyuridine; TdG = 1(3)-[2-deoxy-D-*arabino*-hexopyranosyl]-thymine (deoxyglucosylthymine).

sues *in vivo* by inhibiting the degradation.

IUdR degradation is initiated by two different enzymes: thymidine phosphorylase (EC 2.4.2.4.) (10) and uridine-deoxyuridine phosphorylase (EC 2.4.2.3.) (11, 12). As reported earlier (13), the latter enzyme can be inhibited completely by TdG, whereas the former enzyme is not influenced by this compound. An investigation of the distribution of both phosphorylases by means of the TdG inhibition showed that uridine-deoxyuridine phosphorylase was present in all transplantable tumors of rodents that were examined, and in tissues of dogs, cats, calves, and pigs, whereas thymidine phosphorylase was found in normal tissues of rodents and in normal tissues as well as tumors of man (P. Langen and G. Etzold, unpublished data). Similar results were obtained by Zimmerman (14), who used a different approach. Though it followed from these results that TdG is most probably of no clinical importance, it was nevertheless of interest to study whether this *in vitro* inhibitor is also active *in vivo*, thus increasing IUdR incorporation into DNA. Since their tissues contain uridine-deoxyuridine phosphorylase, cats were chosen as particularly suitable for these experiments.

^{131}I-IUdR was prepared according to Prusoff (15) with some modifications (molar ratio iodine:deoxyuridine = 1:2; preparative thin layer chromatography on Kieselgel G for separation from by-products). Since the IUdR incorporation into DNA is dependent on the growth rate of the tissues, considerable variations between animals of different age may be expected. Therefore we compared only animals of the same litter. IUdR (11 mg/kg, 31 μmoles, 1.5×10^6 cpm) was injected intravenously in 5-week-old cats (mean weight 620 g), anesthetized with hexobarbital. TdG [mixture of 67% α- and 33% β-anomer, prepared according to Etzold and Langen (16)] was given together with the IUdR. Injection volume was 2.0 ml in saline. Two animals were injected only with IUdR, 2 others also received TdG in a molar ratio of TdG:IUdR = 2:1. The fifth animal of the litter received TdG and IUdR in a ratio of 5:1. The animals were killed after 20 hours by hexobarbital. The tissues were extracted twice with 10 volumes of cold trichloroacetic acid for the removal of the acid-soluble ^{131}I compounds and then with 2 volumes of hot trichloroacetic acid for extraction of the nucleic acids. The nucleic acid extracts were analyzed for radioactivity and for DNA content by the diphenylamine reaction (17). Proteins labeled by the ^{131}I released during IUdR degradation (18) remain in the insoluble residue, as has been found in investigations with ^{131}I-labeled fibrinogen (unpublished observations). Moreover, the following control experiments were done to check that the counts in the hot trichloroacetic acid extract were really from IUdR incorporated into DNA. Mice were given i.v. ^{131}I-IUdR or ^{131}I-iodouracil (40 μmoles/kg, 9×10^5 cpm). The latter is not incorporated into DNA (19), and accordingly no significant activity was found in the extracts of intestines and spleens of the animals treated with this compound (less than 3% of the corresponding activity in the IUdR-treated animals). Likewise, in cats given ^{131}I-iodouracil no activity could be found in the extract, whether or not TdG was given simultaneously. We conclude, therefore, that extraction with hot trichloroacetic acid under the above conditions is a suitable method for the estimation of IUdR incorporation into DNA.

The results are shown in Table 1. There was a marked increase of IUdR incorporation into the DNA of bone marrow, spleen and intestine in the animals given TdG:IUdR = 2:1. A still further increase was seen in the animal given TdG at the higher ratio. The different increases in incorporation in the several tissues may reflect their relative phosphorylase activities. Incorporation into liver DNA was not influenced by TdG and showed a considerable variation from animal to animal, which we cannot explain.

In another experiment, not shown here, with 4 cats of one litter, TdG given in a ratio of TdG to IUdR = 2:1 produced a 3–5-fold increase of incorporation of IUdR into the DNA of spleen and intestine (bone marrow not investigated). The observed

TABLE 1
Influence of TdG on the ^{131}I-IUdR incorporation into DNA of various cat tissues[a]

	Bone marrow	Intestine	Spleen	Liver
Without TdG				
Animal 1	790	395	311	636
Animal 2	800	321	295	253
Mean	795	358	303	
TdG:IUdR = 2:1				
Animal 3	6330	1185	1555	1006
Animal 4	5430	1010	1010	416
Mean	5880	1097	1278	
Ratio of Spec. act. with TdG:Spec. act. without TdG	7.4	3.1	4.2	—
TdG:IUdR = 5:1				
Animal 5	11100	1600	1635	321
Ratio of spec. act. with TdG:spec. act. without TdG	14	4.5	5.4	

[a] Values are specific radioactivities (cpm/μmole deoxyribose). A value of 7580 would correspond to 1% replacement of thymine by iodouracil.

effect of TdG at this ratio in cats slightly exceeds that of a 9-fold increase of IUdR dose in mice (51 μmoles/kg and 470 μmoles/kg given i.v. to groups of 5 mice each), which caused a 2.3-fold increase of IUdR incorporation into intestine DNA and a 4-fold increase into spleen DNA (unpublished observations). The effect of TdG in cats on higher doses of IUdR is still unknown. Because of the poor solubility of IUdR, the administration of high doses requires continuous infusion, and it remains to be investigated whether TdG is effective under such conditions.

In mice TdG was found to have no significant effect. This could be expected, because in the mouse the liver, mainly responsible for degradation, contains thymidine phosphorylase.

The improvement of IUdR incorporation into DNA brought about by inhibition of nucleoside phosphorolysis shown in the above experiment emphasizes the potential usefulness of inhibitors of thymidine phosphorylase. They would be expected to improve the therapeutic effectiveness of fluorodeoxyuridine. Such compounds are presently under investigation in our laboratory.

REFERENCES

1. W. H. Prusoff, *Biochim. Biophys. Acta* **39**, 327 (1960).
2. E. G. Hampton and M. L. Eidinoff, *Cancer Res.* **21**, 345 (1961).
3. R. J. Berry and J. R. Andrews, *Rad. Res.* **16**, 84 (1962).
4. A. D. Rotenberg, W. R. Pruce and R. G. Baker, *Brit. J. Radiol.* **35**, 337 (1962).
5. K. H. Clifton, W. Szybalski, C. Heidelberger, F. F. Gollin, F. J. Ansfield and C. H. Vermund, *Cancer Res.* **23**, 1715 (1963).
6. J. P. Kriss, L. Tung and S. Bond, *Cancer Res.* **22**, 1257 (1962).
7. J. P. Kriss and S. Bond, *Biochem. Pharmacol.* **13**, 365 (1964).
8. W. H. Prusoff, *Cancer Res.* **23**, 1246 (1963).
9. A. Čihak and F. Šorm, *Collection Czech. Chem. Commun.* **30**, 324 (1965).
10. M. Friedkin and D. Roberts, *J. Biol. Chem.* **207**, 245 (1954).
11. H. Pontis, G. Degerstedt and P. Reichard, *Biochim. Biophys. Acta* **51**, 138 (1961).
12. T. A. Krenitsky, M. Barclay and J. A. Jaquez, *J. Biol. Chem.* **239**, 805 (1964).

13. P. Langen and G. Etzold, *Biochem. Z.* **339**, 190 (1963).
14. M. Zimmerman and J. Seidenberg, *J. Biol. Chem.* **239**, 2618 (1964).
15. W. H. Prusoff, *Biochim. Biophys. Acta* **32**, 295 (1959).
16. G. Etzold and P. Langen, *Chem. Ber.* **98**, 1988 (1965).
17. Z. Dische, *Mikrochemie* **8**, 4 (1930).
18. W. H. Prusoff, *Biochim. Biophys. Acta* **39**, 327 (1960).
19. W. H. Prusoff, W. L. Holmes and A. D. Welch, *Cancer Res.* **13**, 221 (1953).

Alanine Racemase from *Staphylococcus aureus*: Conformation of Its Substrates and Its Inhibitor, D-Cycloserine

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SUMMARY

D-Cycloserine is a competitive inhibitor of alanine racemase for which its K_i is 100 times smaller than the K_m for either of the substrates, D- and L-alanine. L-Cycloserine, however, does not inhibit this enzyme. A hypothesis is proposed, based on molecular models, that D-cycloserine has the conformation required of the substrates on the enzyme surface. L-Cycloserine cannot have this conformation.

Alanine racemase (1) is the enzyme which interconverts L-alanine and D-alanine in bacteria. This enzyme in *Staphylococcus aureus* is competitively inhibited by D-cycloserine and is one of the targets for the antibacterial action of this antibiotic (2). Further study of this inhibition has led to a hypothesis regarding the conformation of the two substrates on the enzyme surface.

For these studies alanine racemase was purified 100-fold from the supernatant solution prepared after sonic disintegration of *S. aureus*. The pH optimum of the enzyme was 8–9. The K_m for either L-alanine or D-alanine was 4–6 mM when measured in phosphate buffer. The activity of the preparation was not enhanced by the addition of pyridoxal phosphate. By contrast, the enzyme obtained from *S. fecalis* (1) was readily resolved from its cofactor by purification. The *S. aureus* racemase was, however, inhibited by a number of reagents which react with carbonyl groups (Table 1), suggesting the probable presence of pyridoxal phosphate in the enzyme.

TABLE 1

Inhibitor	K_i (mM)	Type of inhibition
NH_2OH	0.012	Competitive
NH_2NH_2	0.10	Competitive
KCN	>10	No inhibition
Semicarbazide	>10	No inhibition
β-Aminoxy-D-alanine	0.04	Competitive
D-Cycloserine	0.05	Competitive
L-Cycloserine	≫10	No inhibition

A surprising result of this study was the finding that, although the K_i for D-cycloserine was about 0.05 mM whether the reac-

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