Tabelle I. Prozentsatz pyknotischer Lymphozyten in Rinde und Mark 8 h nach Bestrahlung mit und ohne Schutzsubstanz

Rinde Dosis	ohne Schutz	+ Cystein	+ Cysteamin	Mark ohne Schutz	+ Cystein	+ Cysteamin
200 R	27,5 ± 1,6	26,3 ± 0,9	24,0 ± 1,0	20,7 ± 1,1	15,8 ± 0,8	16,1 ± 1,3
600 R	50,1 ± 1,2	42,1 ± 1,3	34,1 ± 0,8	36,9 ± 1,7	32,7 ± 1,2	22,3 ± 0,7
800 R	92,0 ± 1,3	61,3 ± 1,2	45,5 ± 1,4	53,0 ± 1,3	41,1 ± 1,1	28,4 ± 1,6

Tabelle II. Anzahl der retikulären Zellen in Prozent aus Rinde und Mark 8 h nach Bestrahlung mit und ohne Schutzsubstanz sowie 8 h nach Injektion von SH-Körper ohne Bestrahlung

Nach Applikation von	Rinde	Mark
physiol. NaCl	0,7 ± 0,2	5,3 ± 0,8
800 R	2,9 ± 0,4	8,0 ± 0,6
800 R + Cystein	8,2 ± 0,4	25,4 ± 1,1
800 R + Cysteamin	5,7 ± 0,3	19,2 ± 0,9
4 mg Cystein	5,4 ± 0,4	26,5 ± 1,4
3 mg Cysteamin	5,8 ± 0,6	20,9 ± 1,1
6 mg Isocystein	6,3 ± 0,5	21,1 ± 1,3

noch im Mark des Thymus eine eindeutige Schutzwirkung an den Lymphozyten erzielt werden. Sie beginnt sich erst nach Applikation von 600 R abzuzeichnen. Nach 800 R wird der Schutzeffekt deutlich, wobei die Pyknosenzahl in der Rinde stärker abnimmt als im Mark. Aus Tabelle I geht ferner hervor, dass die Applikation von Cysteamin einen besseren Schutz bewirkt. Der Prozentsatz pyknotischer Lymphozyten ist noch niedriger und liegt auch unter den Werten, wie sie von TANAKA und RIXON[12] angegeben wurden.

Betrachten wir in diesem Zusammenhang das Verhalten der Retikulumzellen, von denen vermutet wird, dass sie mit der unterschiedlichen Strahlenempfindlichkeit von Rinde und Mark in ursächlichem Zusammenhang stehen, so ergibt sich folgendes: Die in Tabelle II zusammengestellten Werte lassen erkennen, dass die Zahl der Retikulumzellen bei den geschützten Tieren nach Bestrahlung sehr viel stärker zunimmt als bei ungeschützten Tieren. Wie aus Tabelle II weiter hervorgeht, ist die Zahl der Retikulumzellen auch an geschützten, aber unbestrahlten Tieren in ganz entsprechenden Verhältnissen erhöht, obwohl die Lymphozyten keine vermehrte pyknotische Degeneration zeigen. Da in der Literatur mehrfach die Auffassung vertreten wird[13–17], dass eine gesteigerte Aktivierung von RES-Zellen mit einer erhöhten Strahlenresistenz verbunden ist, haben wir auch die Wirkung des nicht schützenden Isocysteins am Thymus untersucht (Tabelle II). Auch diese Substanz bewirkte im Thymus eine Stimulierung der Retikulumzellen. Eine einfache Beziehung zwischen Strahlenresistenz und einer Stimulation des retikulären Systems scheint auf Grund dieser Befunde im Thymus nicht zu bestehen.

Summary. Lymphocytes of the thymus can be protected against early pycnotic degeneration by several SH-compounds. Protective and non-protective SH-compounds induce the same reaction in the non-irradiated mouse, weight loss caused by cell migration, stimulation of the reticulum. A direct relationship between the activity of the reticulum and the radioresistance of lymphocytes was not found.

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[12] Y. TANAKA und R. H. RIXON, Nature *206*, 418 (1965).
[13] E. J. AINSWORTH und M. H. HATCH, Radiat. Res. *13*, 632 (1960).
[14] H. BALNER, L. J. OLD und D. A. CLARKE, Radiat. Res. *15*, 836 (1961).
[15] G. V. C. TAPLIN, C. FINNEGAN, P. NOYES und G. SPRAGUE, Am. J. Roentg. *71*, 294 (1954).
[16] B. W. ZWEIFACH und L. THOMAS, J. exp. Med. *106*, 385 (1957).
[17] K. FLEMMING, CH. FLEMMING und B. GRAACK, Strahlentherapie *131*, 280 (1967).

Studies on Antithiamine Factor(s) in Tissue of Tuna and Goatfish

Numerous studies have dealt with the presence of the enzyme 'thiaminase' in various species of fish[1–4]. This enzyme has been considered to be more prevalent in freshwater than in salt-water fish. Our previous work, however, has shown that tissue from 21 of 30 species of fish found in Hawaiian waters has antithiamine activity[5]. More recently, KUNDIG and SOMOGYI[6] reported the isolation of a non-enzyme antithiamine factor from carp viscera which was identified as hemin or a related compound. The present report concerns chemical properties of an antithiamine factor(s) in several families of tuna and goatfish. These studies indicate that the active agent is not an enzyme.

The fish used in this study were purchased in Honolulu markets and kept frozen until used. Antithiamine tests were made within 1 week after purchase according to the

[1] R. G. GREEN, W. E. CARLSON and C. A. EVANS, J. Nutr. *23*, 165 (1942).
[2] W. YUDKIN, Physiol. Rev. *29*, 389 (1949).
[3] A. FUGITA, Adv. Enzymol. *15*, 389 (1954).
[4] H. F. DEUTSCH and A. D. HASLER, Proc. Soc. exp. Biol. Med. *53*, 63 (1943).
[5] D. M. HILKER and O. F. PETER, J. Nutr. *419*, 89 (1966).
[6] H. KUNDIG and J. SOMOGYI, Int. Z. VitamForsch. *37*, 476 (1967).

procedure described previously[5]. A series of antithiamine activity tests were made at pH values from 0.5–7.0 on yellowfin tuna (*Neothunnus macropterus*) muscle and skipjack tuna (*Katsuwonus pelamis*) muscle and liver (Figure 1). The pH activity curves of muscle of both species were similar with maximum activity at pH 3.0 for the skipjack and 2.5 for the yellowfin tuna. The liver of the skipjack tuna has 2 pH optima, 1.5 and 5.5, which would indicate that the antithiamine substances from the muscle and liver are not the same.

Figure 2 shows the antithiamine activity at different pH values of 5 related families of goatfish; *Parupeneus multifasciatus*, *P. pleurostigma*, *Mulloidichthys auriflamma*, *M. samoensis* and *M. pflugeri*. These pH activity curves were markedly different from those of the tuna. The antithiamine activity of these species of fish at different pH values appears to differ according to the closeness of their relationship. Thus the 3 *Mulloidichthys* have 2 pH optima while the 2 *Parupeneus* have little antithiamine activity throughout the pH range studied.

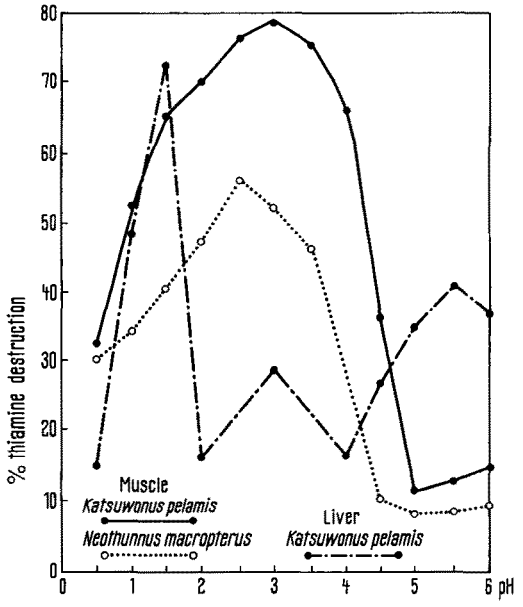

Fig. 1. Antithiamine activity of skipjack tuna muscle, skipjack tuna liver and yellowfin tuna muscle.

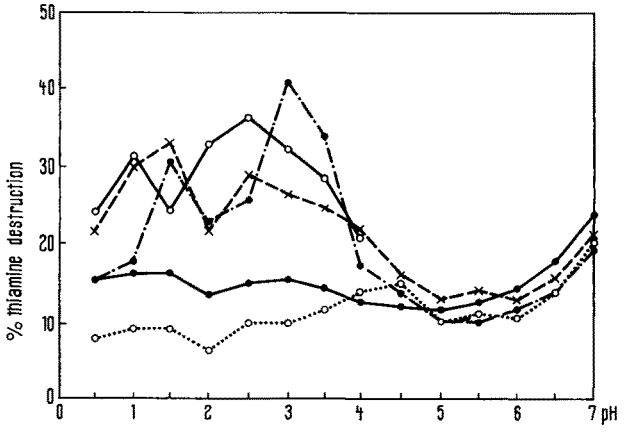

Fig. 2. Antithiamine activity of 5 related families of goatfish: *Parupeneus multifasciatus* ●——●, *P. pleurostigma* ●----●, *Mulloidichthys auriflamma* ×——×, *M. Pflugeri* o——o *and M. samoensis* ●·····●.

A series of tests were done to study the effects of temperature and pre-boiling on the antithiamine activity of skipjack tuna muscle preparations. The incubation temperatures were 5, 23, 38, 75 and 90 °C for each of the following conditions: series 1, homogenate incubated 5 h at pH 3.5 with thiamine (regular); series 2, thiamine added after 5 h incubation period (zero time); series 3, homogenate boiled 10 min, then incubated 5 h with thiamine (pre-boiled).

Greater destruction of thiamine occurred at the higher temperatures, 75 and 90 °C (Table). This activity was not appreciably affected by pre-boiling when the incubation temperature was 75 °C or above but was reduced by pre-boiling when the incubation temperature was lower. Also, the zero time destruction of thiamine was the same as that after a 5 h incubation with thiamine at the 2 higher incubation temperatures. This would indicate that incubating the homogenate at temperatures above 75 °C for a period of time enhances the antithiamine activity and that this activity is not enzymatic.

Further work showed that an antithiamine agent(s) could be released from the soluble protein of the tuna fish homogenate. These studies were made as follows: a 20% homogenate of the tuna fish muscle was prepared in phosphate buffer as previously described[5]. The homogenate was filtered through filter paper, the pH adjusted to 5.5, and then boiled to coagulate the protein; the mixture was then cooled and filtered. There was little or no activity in the filtrate. The precipitated protein was dissolved in phosphate buffer (pH 3.0), incubated for 3–5 h at 75 °C, then cooled and the pH adjusted to 5.5 which coagulated the protein. Tests showed antithiamine activity in the filtrate which increased with longer incubation time. The percent thiamine destroyed was 7, 24 and 60 for 1, 2 and 3 h incubation times respectively. This study is additional evidence that the antithiamine factor is not the enzyme thiaminase as previously reported[5,7,8].

Effect of temperature and pre-boiling on antithiamine activity of a skipjack tuna muscle preparation

Incubation temperature °C	Regular	% Thiamine destroyed pre-boiled	Zero time
5	37	7	20
23	42	7	22
38	44	7	23
75	74	61	74
90	74	60	74

Zusammenfassung. Es wurden Antithiaminwirkstoffe bei Hawaii-Fischen untersucht und gezeigt, dass die Antithiaminaktivität nicht enzymatisch ist.

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[7] This investigation was supported in part by Public Health Service Research Grant No. AM-08531.

[8] Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 962.