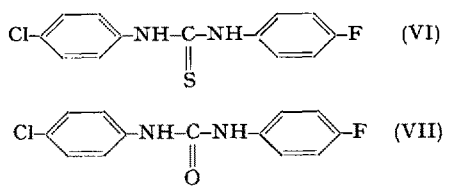
Tableau II. Administration orale

Thiocarbanilides	Dose journalière par 20 g de poids d'animal mg	Survies au 13e jour	Dose tolérée par kg et par jour g
4-Chloro-4'-fluoro-	7	2	0,375
4-Chloro-4'-fluoro-	7	1	0,375
2,5-Dichloro-4'-fluoro-	9	1	1,125
Thiosemicarbazides			
1-(*p*-Fluorophénylaminothioformyl)-	4,5	2	0,225
4-*p*-Chlorophényl-1-(phénylaminothioformyl)	20	0	1,0
4-*p*-Ethoxyphényl-1-(phénylaminothioformyl)-	22	1	1,125
4-*p*-Méthoxyphényl-1-(3,5-dichlorosalicoyl)-	15	0	0,75
4-*p*-Ethoxyphényl-1-isonicotinoyl-	6	0	0,30

Les résultats obtenus avec une cinquantaine de substances, et qui sont résumés dans les 2 Tableaux ci-dessus, confirment l'activité antivirale dans le groupe des dérivés de la thiourée, en particulier des composés fluorés et chlorés. Cette activité, notable lorsque le produit est administré par injection, est beaucoup moins importante par voie buccale. L'influence favorable du groupement $\rangle C=S$ est manifeste, comme il ressort de la chute d'activité observée lorsqu'on passe du 4-chloro-4'-fluorothiocarbanilide (VI) à son analogue oxygéné, le 4-chloro-4'-fluorocarbanilide (VII).



Il est toutefois à noter que deux composés non soufrés, l'acide β-résorcylique et l'acide kojique, montrent une certaine activité. Il est également à remarquer que le 4-chloro-4'-fluorothiocarbanilide possède une faible activité retardatrice sur l'évolution de la polyomyélite expérimentale chez la souris, produite par le virus Lansing (expérience pour laquelle nous remercions M. le Professeur LÉPINE de l'Institut Pasteur de Paris).

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Summary

A large number of sulphur-containing compounds bearing the thiourea group –NH–CS–NH–, including substituted thiocarbanilides, 1-acyl-4-arylthiosemicarbazides, 1-(arylaminothioformyl)-thiosemicarbazides, 4-aryl-1-(arylaminothioformyl)-thiosemicarbazides, and other related substances have been tested for potential antiviral activity. Several of these compounds have been found chemotherapeutically active against influenza virus.

Pitch Discrimination in the Minnow *(Phoxinus laevis)* at Different Temperature Levels

Training experiments have shown that the minnow is able to perceive tones within the range from about 20 to 5000 c/s by using its labyrinth (sacculus and lagena)[1]. Two tones of different frequency are distinguished[2]. The threshold of pitch discrimination at the level of 400 or 800 c/s is about a quarter of a tone (3% frequency difference). Above 1260 c/s, however, there is no pitch discrimination at all[3].

The two main theories explaining pitch discrimination are the place theory[4] and the telephone theory[5]. The place theory seems hardly applicable to the minnow, because any structure suited to serve as a pre-nervous frequency analyser (like the basilar membrane in higher vertebrates) is apparently lacking. The second theory, on the other hand, as modified by WEVER in his volley theory[6], might be applicable[7]. As has been pointed out earlier, a rise in temperature will shorten the refractory period of nerve fibres and therefore increase certain sensory abilities in cold-blooded animals[8]. Similarly, if pitch discrimination in the minnow is really based on the volley principle, a rise in temperature of the animal would effect a rise in its upper limit of pitch discrimination. The following experiments were undertaken in order to test this point[9].

Training method.—According to the method of DIJKGRAAF and VERHEIJEN (1950), whose experiments were made at ± 20°C, blinded minnows were trained to react to a frequency difference of a major third (about 24%) in the range of 400–500 c/s, at room temperature (16°C). By using increasing frequencies, the limit of pitch discrimination was determined. After that the temperature was raised to 25°C and again the limit of pitch discrimination was determined.

[1] K. v. FRISCH und H. STETTER, Z. vgl. Physiol. *17*, 686 (1932).
[2] TH. A. WOHLFAHRT, Z. vgl. Physiol. *26*, 570 (1939).
[3] S. DIJKGRAAF and F. J. VERHEIJEN, Z. vgl. Physiol. *32*, 248 (1950).
[4] H. L. F. v. HELMHOLTZ, 1857 [quoted by E. G. WEVER: *The theory of hearing* (John Wiley and Sons, Inc. New York; Chapman and Hall, Ltd., London, 1949), p. 25].
[5] W. RUTHERFORD, 1886 [quoted by E. G. WEVER (1949), p. 77].
[6] E. G. WEVER, 1949.
[7] O. LÖWENSTEIN and T. D. M. ROBERTS, J. Physiol. *114*, 471 (1951).
[8] E. D. ADRIAN, K. J. W. CRAIG, and R. S. STURDY, Proc. roy. Soc. [B] *125*, 435 (1938).
[9] Preliminary results were obtained by W. VAN DER LEE in this laboratory (unpublished records).

Every experiment was started by repeatedly offering the fish an interrupted tone of a given frequency. The fish, being never rewarded, soon gave up reacting to this "neutral-tone signal". After at least a quarter of an hour, every second tone of the neutral-tone signal was suddenly replaced by a tone of another frequency without interrupting or altering the rhythm of the signal. The reaction of the animal was observed (observation *P*), the fish rewarded by food and, still without altering the rhythm, the "training signal" brought back to the neutraltone signal. Sequence of tones:

neutral training neutral

After waiting till the animal was quiet again, the next observation could be started.

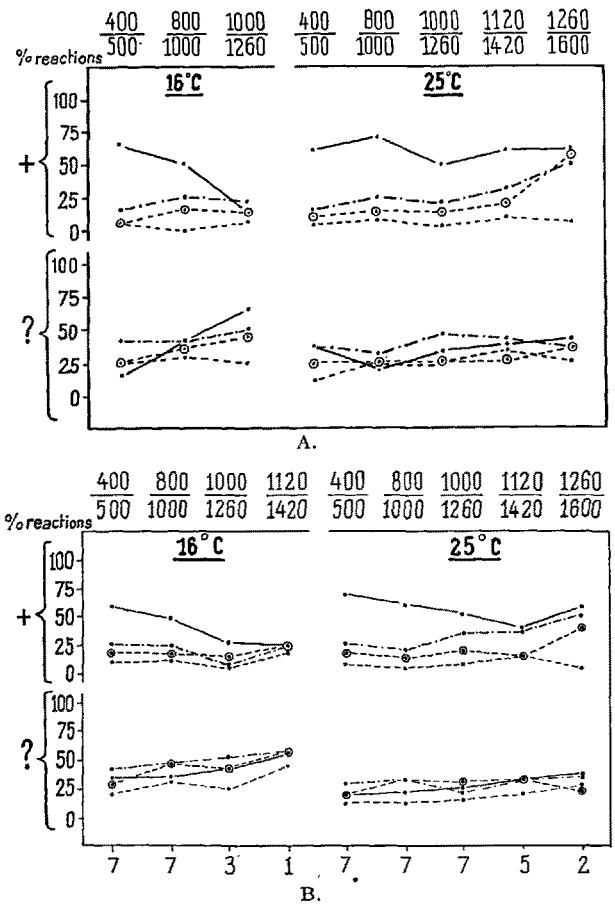

Fig. 1. Reactions of trained minnows to pitch at different temperatures.—*A* Results of one single fish. *B* Mean of results of all seven fishes tested.—*Ordinate:* Percentage of positive (+) and doubtful (?) reactions (100% = 20 observations). *Abscissa:* Training stimulus (frequency difference in c/s: major third).

·——·——· Frequency reactions (P).
·—·—·—·—· Intensity reactions (C_i).
⊙---⊙---⊙ Neutral-tone reactions (C_n).
·-----·----- Spontaneous "reactions" (C_a).

The following control observations were made:

(1) C_i: Tests with intensity difference (loudness of neutral-tone signal alternating low and high), because intensity differences between the two tones of the interval might be of importance[10].

(2) C_n: Reactions on neutral-tone signal were observed.

(3) C_a: Spontaneous "reactions" in the absence of sound were observed.

Apparatus.—The same apparatus as described by DIJKGRAAF[11] was used with slight alterations.

Method of observation.—About 50% of the observations were made by the author with one assistant. The latter handled the apparatus and gave instructions whenever an observation period began. In addition he put down the records of the observer and, afterwards, gave the signal when to feed. In this way the observer did not know, while observing, if a reaction could be expected. These observations and those made by the author, both handling the apparatus and observing at the same time, agreed satisfactorily. At all frequencies examined for every fish fit for use twenty observations were made for each *P*, C_i, C_n and C_a. The reactions were recorded as follows: + = the typical feeding reaction; ? = agitation, but no typical feeding reaction; − = no reaction at all. The reactions of the animals during the three control observations were recorded in the same way. Consequently, the more + reactions in answer to a frequency difference as compared with the + reactions in answer to the controls C_i, C_n and C_a, the better the frequency discrimination.

Results.—The results of one single fish and the mean of the results of all seven fishes are shown in Figures 1*A* and *B* respectively. In both graphs the experimental results at 16°C will be found on the left hand side, and those at 25°C on the right hand side. In Figure 1*B* the figures at the bottom of the diagram serve to show the number of animals fit for use (and used) for each interval. Because of the individually different performances this number decreased with rising pitch.

The following conclusions seem justified:

(I) The upper limit of pitch discrimination in minnows is raised from 800–1260 c/s at 16°C to 1260–1420 c/s at 25°C. Whereas all 7 fishes tested reacted to a frequency difference of 1000–1260 c/s at 25°C only 3 of them did (less well) at 16°C.

(II) This change is not due to a general activation of these coldblooded animals by the higher temperature: (i) the graphs of the *P*+ reactions are either tilted or at a higher level at 25°C compared with those at 16°C; (ii) the graphs of C_i, C_n and C_a are at the same or at a lower level at 25°C as compared with those at 16°C respectively (in Figure 1*B* only figures from equal numbers of fish should be compared). A general activation would show a rising of all graphs at 25°C.

The outcome of these experiments supports the view that pitch discrimination in fish might be based on the volley principle.

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Zusammenfassung

Aus früheren Untersuchungen war bekannt, dass die Elritze (*Phoxinus laevis*) imstande ist, Töne nach ihrer Frequenz (Tonhöhe) zu unterscheiden, jedoch nur im Frequenzgebiet bis zu etwa 1260 Hz, nicht darüber (die obere Hörgrenze liegt bei etwa 5000 Hz). Es wurde geprüft, welchen Einfluss Wechsel der Temperatur auf die Lage dieser oberen Schwelle der Frequenzunterscheidung hat. Sie lag für die einzelnen Versuchstiere bei

[10] S. DIJKGRAAF and F. J. VERHEIJEN, Z. vgl. Physiol. *32*, 248 (1950). – D. POGGENDORF, Z. vgl. Physiol. *34*, 222 (1952).

[11] S. DIJKGRAAF, Z. vgl. Physiol. *34*, 104 (1952).

16°C zwischen 800 und 1260 Hz; bei 25°C zwischen 1260 und 1420 Hz, also höher. Der Unterschied beruht nicht auf grösserer Lebhaftigkeit oder Reaktionsbereitschaft der Fische bei der höheren Temperatur. Das Ergebnis steht im Einklang mit der Vermutung, dass die Tonfrequenzunterscheidung bei Fischen nach dem «volley principle» (WEVER) erfolgt.

PRO EXPERIMENTIS

Papierchromatographische Ultramikro-Alkalimetrie

Die von WIELAND und Mitarbeitern[1] ausgearbeitete und zur quantitativen Bestimmung kleinster Mengen (etwa 1,5 γ) von Aminosäuren benützte retentiographische Methode lässt sich, wie wir fanden, auf die Identifizierung und Bestimmung anorganischer Ionen übertragen. Am Beispiel der Ultramikrobestimmung anorganischer Basen sei die Arbeitsweise beschrieben, mit deren Hilfe es gelingt, noch etwa 2 γ OH-Ionen (das sind ca. 5 γ NaOH) zu erfassen, eine Menge, die auf trimetrischem Weg mit Farbindikatoren nicht mehr bestimmbar ist[2].

Die auf OH' zu prüfende Lösung wird in einer Reihe mit Kontrollösungen, die bekannte Mengen der Base enthalten, auf der Startlinie aufgetragen. Die Entwicklung des Retentions-Chromatogramms erfolgt mit einem HCl-haltigen Lösungsmittel; 96%iger, mit 1% Benzol denaturierter Alkohol erwies sich als geeigneter als Dioxan oder Tetrahydrofuran. Die verwendete HCl-Konzentration – wir arbeiteten mit HCl-Konzentrationen von 0,1 bis 0,01%, das sind 1,3 bis 0,13 cm³ 2n HCl auf 100 cm³ – soll entsprechend der zu messenden OH'-Menge, die in einem orientierenden Vorversuch festgestellt werden kann, gewählt werden, die Laufzeit möglichst kurz (vgl. Tabellen I und II). Wie Versuche zeigten, ist Temperaturkonstanz erforderlich.

[1] TH. WIELAND und E. FISCHER, Naturwissenschaften *35*, 29 (1948). – TH. WIELAND, Angew. Chem. *60*, 313 (1948). – TH. WIELAND und L. WIRTH, Angew. Chem. *63*, 171 (1951). – TH. WIELAND und U. FELD, Angew. Chem. *63*, 258 (1951). – Vgl. auch B. ERDEM, B. PRIJS und H. ERLENMEYER, Helv. chim. Acta *38*, 267 (1955).

[2] Vgl. P. L. KIRK, *Quantitative Ultramicroanalysis* (J. Wiley & Sons/Chapman & Hall, New York-London 1951).

Tabelle I
NaOH
Lösungsmittel: 98 cm³ 96%iger Alkohol + 2 cm³ Wasser

Chromatogramm Nr.	1	2	3	4	5	6	7
HCl-Konzentration %	0,05	0,05	0,05	0,025	0,025	0,01	0,01
Temperatur °C	25	30	30	25	30	25	30
Laufzeit h	1	½	1	1½	1	2½	1
Gefunden cm² je. *a* 5 γ				0,68	0,46	0,45	0,71
b 10 γ	1,05	0,69	0,64	1,32		0,93	1,34
c 15 γ	1,48	1,24	1,09		1,52	1,43	
d 20 γ	2,07	1,60	1,34	2,77	1,94		
cm²/ε *a*				5,44	3,68	3,60	5,68
b	4,20	2,76	2,56	5,28		3,72	5,36
c	3,92	3,28	2,88		4,08	3,84	
d	4,14	3,20	2,68	5,54	3,88		

Tabelle II
Na_2CO_3
Lösungsmittel: 98 cm³ 96%iger Alkohol + 2 cm³ Wasser

Chromatogramm Nr.	8	9	10	11	12	13	14
HCl-Konzentration %	0,05	0,025	0,025	0,025	0,025	0,01	0,01
Temperatur °C	25	25	30	30	30	25	30
Laufzeit h	1	1½	½	1	1	2½	1½
Gefunden cm² je. *a* 5 γ		0,54					
b 10 γ	0,62	1,10		0,54		1,07	1,50
c 15 γ	1,00	1,64				1,59	2,25
d 20 γ	1,37		1,13	1,14	1,40	2,08	
e 30 γ			1,74	1,65	2,06		
cm²/ε *a*		5,83					
b	3,29	5,83		2,86		5,67	7,95
c	3,53	5,80				5,62	7,95
d	3,63		3,00	3,00	3,71	5,51	
e			3,07	2,92	3,64		