Table II shows the effect of thiamine deficiency on these enzyme activities in intestinal mucosa. Both enzyme activities decreased markedly in thiamine deficiency and were restored to the control level after thiamine-HCl (experiment 1). In addition, TDPase was restored completely and only ALPase was restored partly after vitamin D_3 which is known to induce an increase of Ca^{++} absorption and intestinal ALPase in rachitic animals[10], (experiment 2). The corresponding changes of TDPase and ALPase activities in response to thiamine deficiency, coupled with the similar distribution of two enzymes in small intestine, led us to consider that the two enzyme activities represented different measures of the activity of a single enzyme. To investigate this point, we carried out the purification of ALPase from intestinal mucosa in normal rats by the method of SAINI et al.[11], modified in that enzymically active fractions from each column chromatography were concentrated by ultrafiltration instead of ethanol precipitation.

Table III. Results of the enzyme purification steps

Steps	Protein (mg)	Specific activity (µmoles/mg protein/min)		Ratio a/b
		ALPase[a]	TDPase[b]	
Mucosal homogenate	7260	1.73	0.95	1.82
n-Butanol extract	537	8.73	8.50	1.03
Ethanol precipitate	245	30.7	11.8	2.60
DEAE cellulose fraction	81	148	76.2	1.94
Sephadex G-200 fraction	28	175	190	0.92
DE-32 cellulose fraction	19	187	217	0.86
DE-32 cellulose fraction	2	588	482	1.22

TDPase was purified together with ALPase (Table III) and *p*-nitrophenylphosphate (P-NPP) behaved as a competitive inhibitor of TDP hydrolysis (Figure); Ki value obtained by the method of DIXON[12], using data shown in the Figure, was about 0.1 mM. The value is in agreement with K_m value of P-NPP hydrolysis (K_m 0.14 mM). Furthermore, two enzyme activities in the purified material showed a similar response to heat denaturation and to L-phenylalanine, a stereospecific inhibitor of intestinal ALPase[13] (data not shown). These results strongly suggest that TDPase is identical with ALPase in the intestine.

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Reactions of the Goldfish (*Carassius auratus auratus* L.) to Quantified Mechanical and Thermal Stimuli[1]

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Summary. Goldfish can differentiate between mechanical and thermal stimuli components in combined stimulations. Reactions to heated thermode ($\Delta T = +3$ °C) differ from those to isotherm thermode by up to 30%. Additional pretension (0.5 *p*) increases the reaction more than double; at simultaneously varied temperature ($\Delta T = +3$ °C and $+5$ °C), there is a further increase in reaction of 25% and 35%. The significance for the organism of the two stimuli components is discussed.

Bony fish can be conditioned with different methods to react to temperature. Besides, fish are capable of an exact temperature selection within a temperature gradient[3]. In addition, DIJKGRAAF[4] found out that minnows (*Phoxinus laevis*) and catfish (*Ameirus nebulosus*) can be conditioned to water jets of different temperatures, even if the lateral line system is eliminated. During further investigations of the distribution of thermal sensitivity over the skin, BARDACH[5] and SERBENYUK and MANTEIFEL[6] showed, by means of stimulation with thermodes, that the entire body surface of the fish is involved in the sensation of temperature.

In general, thermal investigations of aquatic vertebrates had thermal stimuli coupled with mechanical stimuli. Up to now, research on the temperature sense has not sufficiently taken into account that, in this combination, mechanical stimulation becomes effective simultaneously. The obvious connection of the two stimuli modalities has also been confirmed by our investigations where no specific thermoreceptors could be found in fish

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skin[7,8]. As in the natural biotope the stimuli mainly appear in the same combination described above, the question arises to what extent the organism is able to perceive and to use the two modalities differently and independently.

In the present experiments, goldfish were subjected to mechanical and thermal stimuli which were quantifiable separately. 3 animals (length 10–12 cm) were tested over a period of $1^{1}/_{2}$ years. In an isolated aquarium, the fish were conditioned to come to a particular place through light-food conditioning. Temperature in the aquarium was kept at steady 20 °C by constant-temperature control. Food was given through an illuminated glass tube, light served as a signal stimulus. The feeding spot was equipped in such a way that the animals while being fed had to touch a thermode with the body within an experimental chamber (lucite). The temperature of the thermode and its mechanical pre-tension could be varied separately. In this way the fish became accustomed to the spot conditioning as well as to the light touch. Pressing the front part of the thermode was regarded as reaction (measured in p). The front surface of the electrically heated thermode was calibrated with a thermistor. This slightly domed surface, directed at the animal, measured 80 mm^2, the area effectively touched about 10 mm^2.

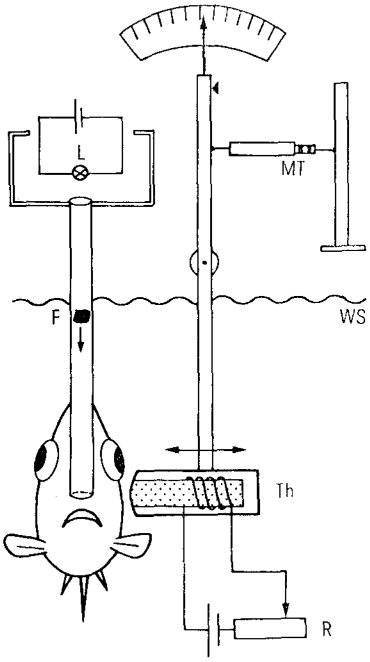

Fig. 1. Schematic drawing of the experimental arrangement for mechanical and thermal stimulation of the conditioned fish when fed through an illuminated glass tube. F, food; L, lamp in an isolated box; MT, steel spring for mechanical pre-tension of the thermode; R, resistance for varying temperature of the thermode; Th, thermode; WS, water surface.

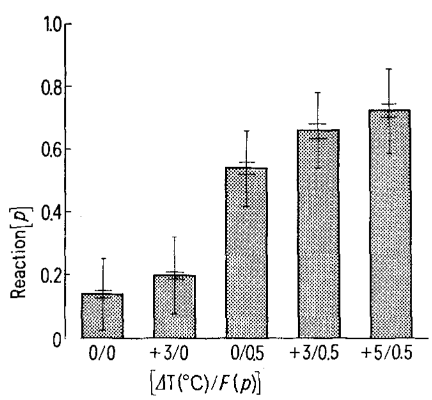

Fig. 2. Reactions to various mechanical and thermal stimuli combinations. Reaction: Force (p) of the fish pressing against the thermode; numbers at the base of the columns represent the different stimuli combinations. Long vertical lines at the top of columns = SD (± s); lines close to the top of columns = ± SEM.

Four successive feedings were carried out every 3rd day with constant stimulation over 4–6 weeks. The interval between 2 feedings was about 2 min. The food consisted of small slices of a mixture of dry food and gelatine. The whole arrangement was hidden behind a wall so that the fish could not see the experimentator. After only 4–6 successful spot conditionings, could the series of investigation be begun.

Figure 1 demonstrates the apparative arrangement schematically. 4 different stimuli combinations were used in the course of investigation: 1. Isotherm thermode (thermode temperature = water temperature), pre-tension of the thermode = 0 p. 2. Heated thermode (thermode temperature = water temperature + 3 °C), pre-tension of the thermode = 0 p. 3. Isotherm thermode, pre-tension of the thermode = 0.5 p. 4. a) Heated thermode (thermode temperature = water temperature + 3 °C), pre-tension of the thermode = 0.5 p. b) Heated thermode temperature = water temperature + 5 °C), pre-tension of the thermode = 0.5 p.

During the course of the experiments, reactions showed a small decreasing tendency. Slight growth of the fish did not have any serious influence.

The following results can be stated. The experiments without a mechanical pre-tension show a reaction which is low at isotherm as well as at heated thermode. On the average, the fish presses to about 0.2 p. But a distinct increase in reaction can be observed when the thermode is heated. The rise in reaction at the heated thermode of $\Delta T = +3\,°C$ already signalizes that the animal senses the higher temperature, because only the parameter 'temperature' is changed. The light pressing of the thermode corresponds with observations made by BARDACH[5] who also noticed a slight thigmotaxis reaction at conditioning. When the series with isotherm and heated thermode are repeated, the same effects can be seen. The results of a stronger reaction with heated thermode are rather conclusive, since the difference of the stimulus is above the threshold found by BARDACH[5] and SERBENYUK and MANTEIFEL[6] at Cyprinids (2 °C difference temperature at 2 mm^2 resp. 0.9 °C at 5 mm^2 of skin area at stimulation with a thermode).

Mechanical pre-tension of the thermode of $F = 0.5\ p$ clearly increases the reaction. This reaction is about $2^{1}/_{2}$ times stronger than the values obtained without any pre-tension; it amounts to about 0.5–0.6 p. The additional pressing of the fish corresponds fairly well with the force of the thermode itself. A possible interpretation of this effect is the effort of the fish to compensate the drift produced by the pressure when coming to the feeding spot (Figure 2).

The next series of investigation with same mechanical pre-tension and additionally heated thermode show re-

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actions increasing further at higher temperatures. Compared to reactions at isotherm conditions, these increases amount to about 25% when the thermode is 3 °C warmer, and 35% at 5 °C more. Here too, subsequent experiments with the same mechanical pre-tension and isotherm thermode show the values initially received. Observations by BARDACH[5], who did not get any reactions to isotherm touches after temperature conditioning, confirm this. As the mechanical force of the stimulus was not changed here, and the reaction increased right after the beginning of the new stimuli combination, this change of reaction must be associated to the changed temperature.

The reactions of a single animal to the various stimuli combinations are represented in Figure 2. Each column consists of about 50 measurements.

On the basis of these results, one has to inquire about the significance of the temperature component in these stimuli combinations. The stronger reactions with heated thermode are underlined by the fact that, according to the t-test by Student[9], the differences between the 4 pairs of stimuli combinations are significant (0.27%-level), except for the last one (see Figure 2).

According to electrophysiological investigations of the fish skin[7], mechanical stimulation and simultaneous heating lower the reaction within the skin nerve. This corresponds to a cold-receptor-characteristic of those mechanoreceptors. It can be assumed that the same characteristics are present in behaviour experiments. Therefore the increased reaction of the goldfish can be seen as compensation of the reduced skin nerve activity. However, this effect has to be coupled with a simultaneous temperature perception, since, 1. there is no evidence so far that there is a special receptor system for temperature in fish, and 2. they can still be well conditioned to small temperature differences as low as 0.05 °C[10,11]. It is interesting that stronger pressing of the fish at heated thermode corresponds with observations of WEBER[12] who noted that warm subjects seem lighter for man.

In which way temperature information is effectively perceived and processed in the CNS has to be clarified by further investigations.

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The Effect of Sugar Nutrition of in vitro Pollinated Placentae on Seed Set and Dormancy in *Nicotiana tabacum* L.

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Summary. The substitution of sucrose in the nutrient medium for in vitro pollinated placentae by glucose and fructose favours seed formation and considerably enhances the germination of seeds just attaining maturity. This effect of invert sugar was wholly suppressed in the mixture with two parts of sucrose.

The pollination of excised placentae cultivated on artificial media results in ovule fertilization and viable seed formation[1,2]. In certain cases, the seeds formed in vitro germinate directly on placentae[2–6]. However, very often no exact data concerning the period between seed maturation and the beginning of germination are given. In our experiments with *Nicotiana tabacum*, seed germination in situ varies from experiment to experiment and in some cases all seeds dry out and pass to a dormant state as under natural conditions. For test-tube fertilization, sucrose has been the only sugar component of the medium so far used. Its substitution by glucose and fructose is shown here to affect seed formation and seed germination on placentae.

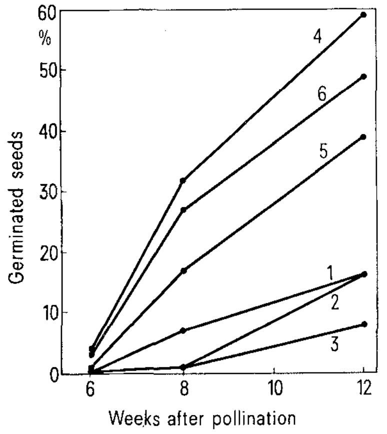

Fig. 1. The course of germination of seeds formed on excised placentae cultivated on various media: 1 and 2, media with sucrose (0.15 M); 3, medium with sucrose (0.1 M) + glucose (0.025 M) + fructose (0.025 M); 4, medium with glucose (0.075 M) + fructose (0.075 M); 5, medium with glucose (0.1 M) + fructose (0.05 M); 6, medium with glucose (0.05 M) + fructose (0.1 M). All media were sterilized under normal pressure, except the medium 2 which was autoclaved.

Material and methods. Experimental plants of *Nicotiana tabacum* L., cv. Samsun were grown in free soil in a glasshouse. The techniques of excision, pollination and cultivation of placentae were the same as described earlier[7]. The basal medium prepared according to NITSCH[8] was supplemented by White's vitamins and 0.05% casein hydrolysate, the final concentration of sucrose being 0.15 M[2]. The medium was either autoclaved for 20 min at 0.15 MPa or sterilized under normal pressure successively

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