Mode of action of niclosamide in various parasitic helminths and host isolated tissues

Preparation	Concentration of niclosamide (M) 3×10^{-9}	3×10^{-8}	3×10^{-7}	3×10^{-6}	3×10^{-5}
Cestoda					
Dipylidium caninum		▼	▼△	○	
Diphyllobothrium erinacei (plerocercoid)				▼	■
Diplogonoporus grandis		▼	△	○	
Trematoda					
*Metagonimus yokogawai**		▼	▼	■	
Paragonimus westermani		△	○	■	
Fasciola hepatica		△	△	○	
*Schistosoma japonicum**				△	○
Nematoda					
Angiostrongylus cantonensis	▼	▼	△	○	
Dirofilaria immitis			△	○	
Trichuris vulpis					○
Toxocara canis					△
Ancylostoma caninum			△	△	■
Frog rectus (guanidine-induced twich response)		△	○		
Mouse ileum		▼	■		

*Tested by the visual observation method. Inhibitory effect: ▼, slight affection; ■, complete paralysis. Excitatory effect: △, slight affection; ○, complete spastic paralysis.

cits its spastic and/or paralytic action through a neuropharmacological mechanism including acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) in *D. caninum*[20]. Since Strufe and Gönnert[19] suggested that niclosamide acts on oxygen uptake and oxidative phosphorylation through its action on ATPases, the neuropharmacological effects of this compound may also be elicited through its action on the same sites and be related to the release of neurotransmitters.

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Studies on chemotherapy of parasitic helminths (IX). Effects of praziquantel on the motility of various parasitic helminths and isolated host tissues

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Summary. Praziquantel (PQ) caused spastic and/or paralytic actions on the motility of various parasitic helminths and isolated host tissue preparations, through a neuropharmacological mechanism.

Praziquantel (2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydro-2H-pyrazino [2,1a] isoquinolin-4-one, PQ) is a newly developed anthelmintic with a broad spectrum against trematodes and cestodes. Since Gönnert and Andrews[1] reported antischistosomal effects of PQ, there have been many reports regarding the in vivo effects of this compound in animal[1-3] and clinical[4-8] experiments against trematodes such as *Schistosoma mansoni*[1,2,4], *S. haematobium*[2,5], *S. japo-*

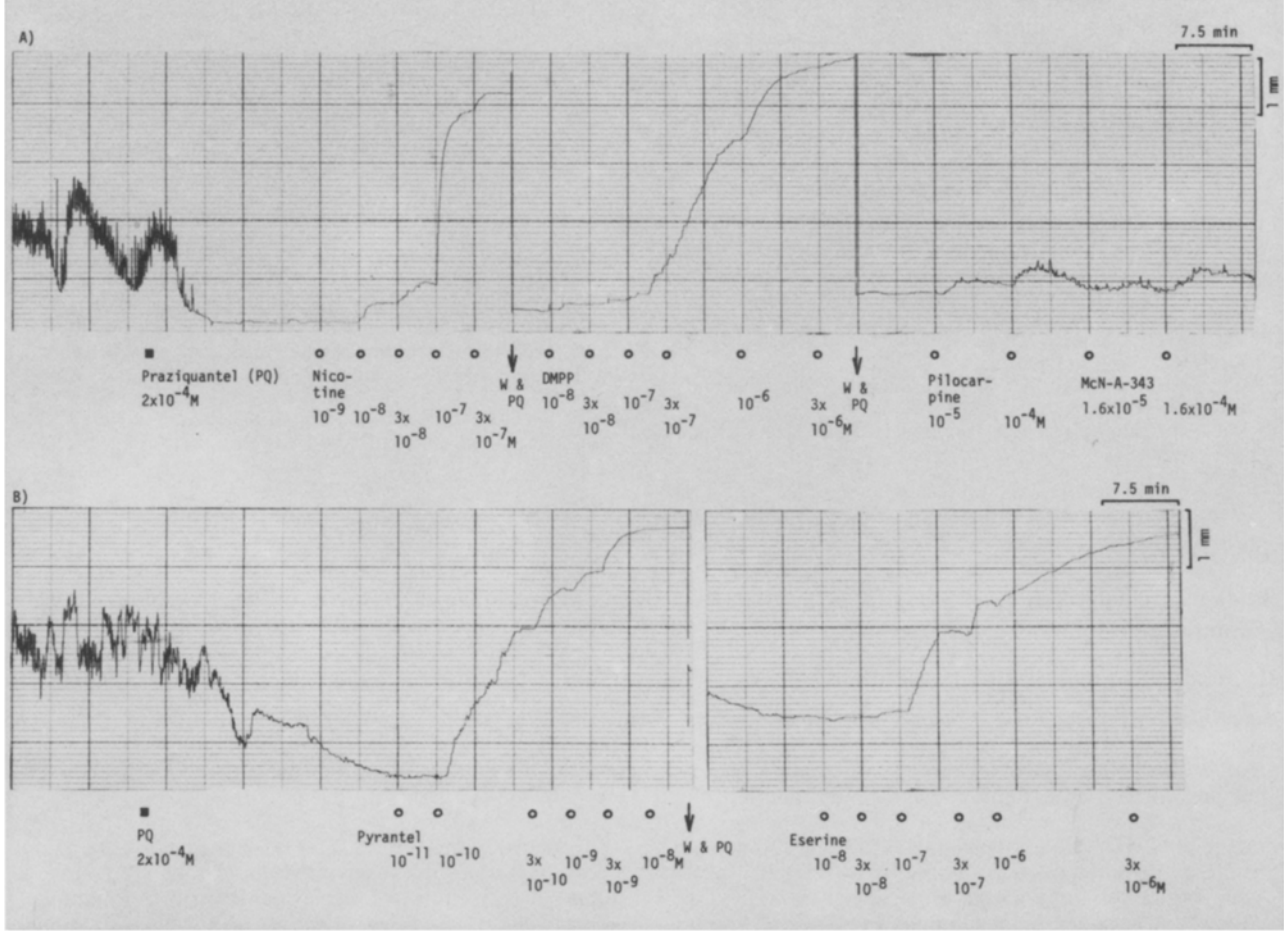
nicum[2,6], *Clonorchis sinensis*[7,8] and *Paragonimus westermani*[3,8]. There have also been reports describing in vivo effects against juvenile and adult cestodes[9,10]. Furthermore, some investigators have discussed the mode and mechanism of action of PQ in experiments in vitro. Using a visual observation method, Thomas and Andrews[9] showed spastic paralysis of strobila of *Hymenolepis diminuta* by PQ, and also a rapid onset and reversibility of the action of this compound. As to a mechanism of action, some investigators have reported an inhibition of aspects of energy metabolism[9-12] such as glucose-[9] and oxygen-[12] uptake, some have reported influences on ion-movement such as Na^+, K^+ and Ca^{2+} [13,14], and others have described morphological changes in the reproductive system[11] and mitochondria[12].

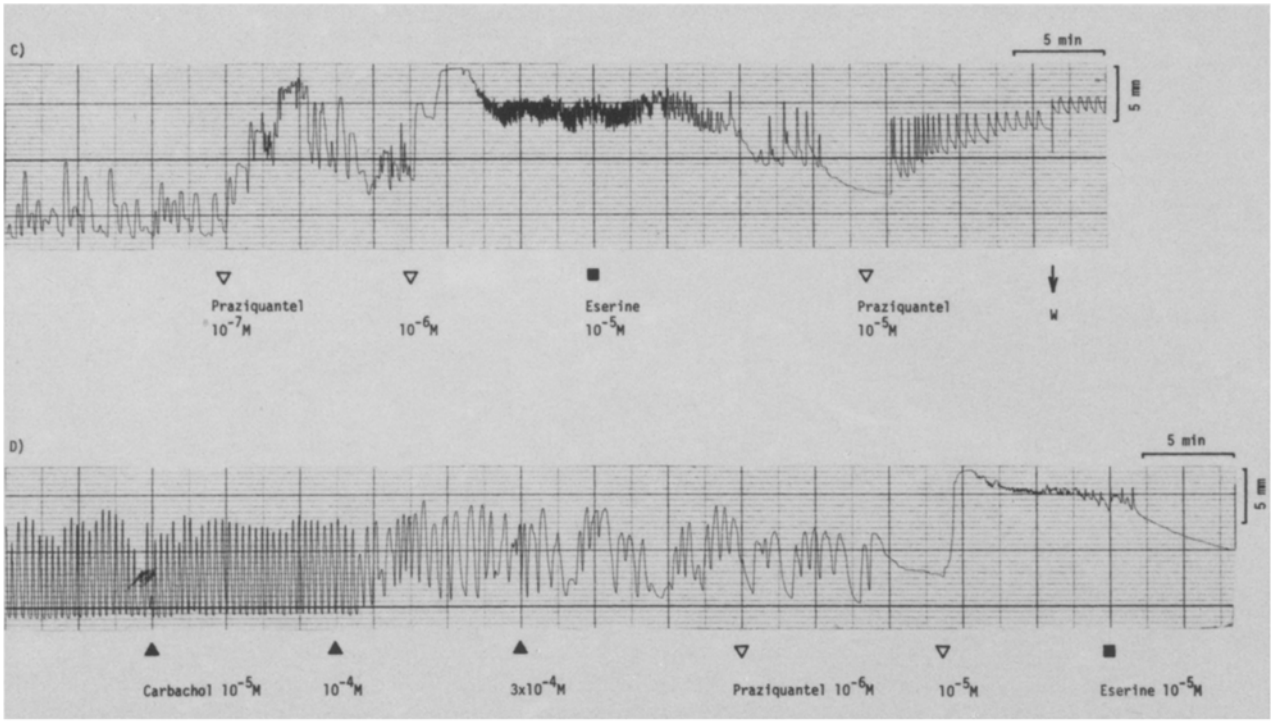
In the present study, in vitro effects of PQ on the motility of various parasitic helminths and isolated host tissues were comparatively studied, and a mechanism of action of this compound was also studied neuropharmacologically.

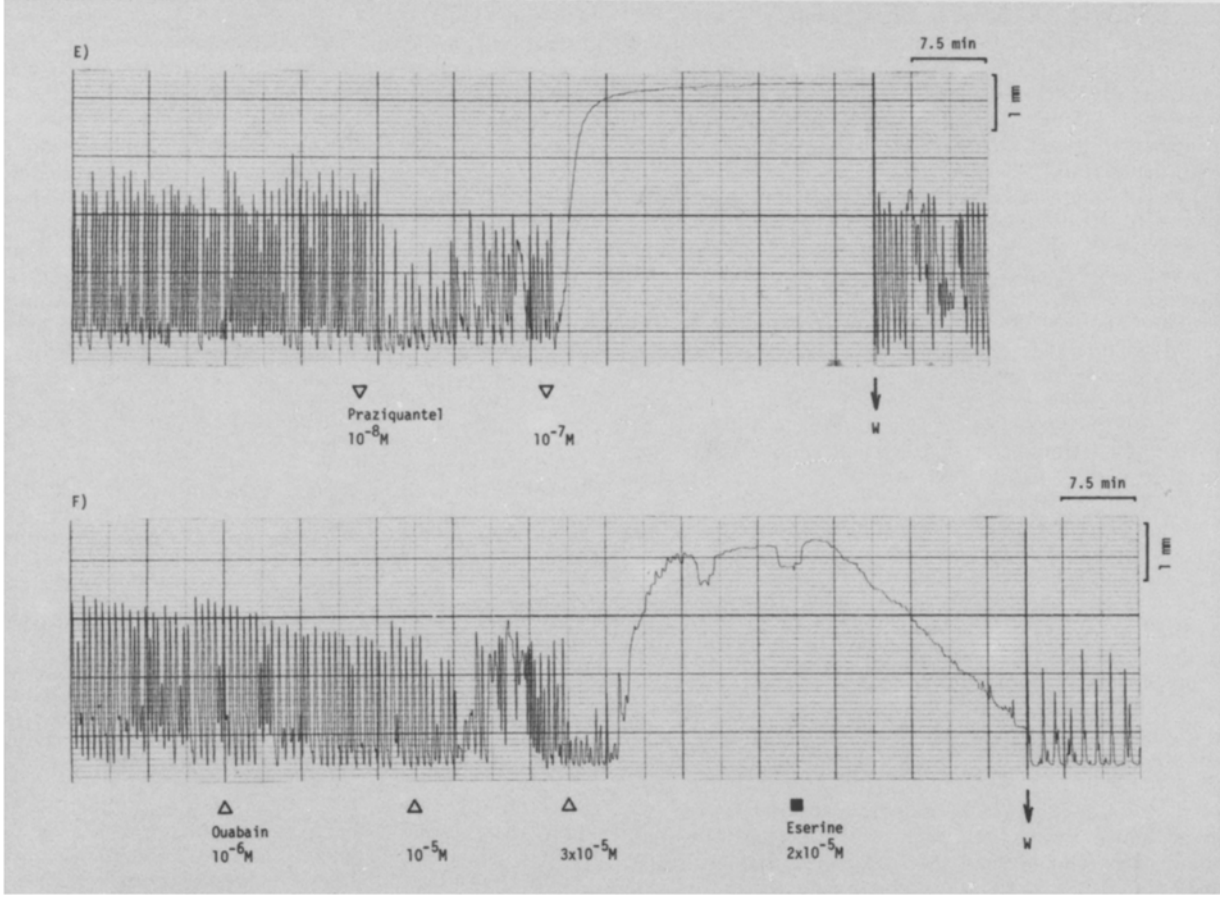
Materials and methods. Worms were obtained from the animals sacrificed at the Hamamatsu slaughterhouse or from those experimentally infected in our laboratory. The isotonic transducer and visual observation methods previously described[15,16] were used.

Results and discussion. In the present in vitro study, PQ showed spastic and/or paralytic actions against all preparations, including parasitic cestodes, trematodes, nematodes, and isolated host tissue preparations (table). Moreover, many of the cestodes and trematodes were most susceptible to PQ, that is, the motility was paralyzed spastically at 10^{-8} M (3.1×10^{-9} g/ml) in *Taenia pisiformis* and at 10^{-7} M in *Dipylidium caninum* and *P. westermani*, and at 10^{-6} M in *Diplogonoporus grandis*, *Metagonimus yokogawai* and *S. japonicum*. At a concentration of 10^{-5} M, the motility of plerocercoids of *Diphyllobothrium erinacei* was paralyzed, while the guanidine-induced twitch response in the isolated frog rectus preparation was paralyzed spastically. At a higher concentration of 10^{-4} M, the motility of *Fasciola hepatica* was paralyzed spastically, while that of *Ancylostoma caninum* and the isolated mouse ileum preparation was paralyzed. On the other hand, the motility of nematodes such as *Angiostrongylus cantonensis* and *Trichuris vulpis* was less susceptible to this compound, and was paralyzed at the concentration of 2×10^{-4} or 3×10^{-4} M. Thus, in vitro effects of PQ in this experiment agreed well with the results as to in vivo effects previously reported[1-10].

In our comparative and systematic studies on drug actions between various parasitic helminths and isolated host tissue preparations, we[17] have suggested that *A. cantonensis*, our nematodal model, has a nervous system similar to that reported in *Ascaris suum*[18], whose exitatory and inhibitory neurotransmitters are presumably acetylcholine (ACh) and γ-aminobutyric acid (GABA), respectively. It has also been suggested by us that both cyclophyllidean cestodes (*D. caninum*, our cestodal model)[19] and pseudophyllidean cestodes (*D. grandis*)[20] have a nervous system similar to those reported in trematodes[21] such as *S. mansoni* and *F. hepatica*, whose excitatory and inhibitory neurotransmitters are probably 5-hydroxytryptamine (5-HT) and ACh, respectively. Therefore in this study we undertook a neuropharmacological investigation of the action of PQ; though biochemical, physiological and morphological changes due to this compound have also been reported[9,11-14].







Effects of praziquantel (PQ) and other neuropharmacological agents on the motility of *Angiostrongylus cantonensis* (A, B), *Diplogonoporus grandis* (C, D) and *Paragonimus westermani* (E, F). Relationships between PQ or ouabain and cholinergic agonists were shown. DMPP: 1,1-dimethyl-4-phenylpiperazinium iodide; McN-A-343: 4-(m-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride; W: Washed by Tyrode's solution; W & PQ: washed by Tyrode's solution and then treated with praziquantel (2×10^{-4} M).

Mode of action of praziquantel on various parasitic helminths and isolated host tissue preparations

Preparation	Concentration (M) 10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	$2-3\times10^{-4}$
Cestoda							
Dipylidium caninum		▼△	○				
Taenia pisiformis	△	○					
Diplogonoporus grandis			△	○			
Diphyllobothrium erinacei (plerocercoid)				▼	■		
Trematoda							
*Metagonimus yokogawai**			△	○			
Paragonimus westermani		▼	○				
Fasciola hepatica					△	○	
*Schistosoma japonicum**			△	○			
Nematoda							
Angiostrongylus cantonensis						△	■
Trichuris vulpis							■
Ancylostoma caninum					▼	■	
Toxocara canis							■
Ascaris suum						▼	■
Dirofilaria immitis						△	■
Frog rectus (guanidine-induced twitch response)				▼△	○		
Mouse ileum					▼	■	

*Tested by the visual observation method. Inhibitory effect: ▼, slight affection; ■, complete paralysis. Excitatory effect: △, slight affection; ○, complete spastic paralysis.

In preparations of *A. cantonensis* paralyzed with PQ (2×10^{-4} M), remarkable contractions were caused by cholinergic agonists which have nicotinic properties, such as nicotine (10^{-8}–3×10^{-7} M), DMPP (10^{-7}–3×10^{-6} M), eserine (10^{-7}–3×10^{-6} M) and pyrantel (10^{-10}–10^{-8} M), but not caused by those which have muscarinic properties, such as pilocarpine (10^{-5}–10^{-4} M) and McN-A-343 (1.6×10^{-5} M) (fig., A, B). On the other hand, in preparations such as *D. caninum, D. grandis, P. westermani* and *F. hepatica,* the action of PQ was antagonized by eserine (10^{-5} M) and carbachol (10^{-4}–3×10^{-4} M). The figure (C and D) shows effects of PQ, eserine and carbachol on the motility of *D. grandis.* The motility was remarkably stimulated by PQ (10^{-6} M) in rate and tone, and the stimulated preparation was paralyzed by the addition of eserine (10^{-5} M). However, the paralyzed preparation showed contractive activity again on the further addition of a higher concentration of PQ (10^{-5} M). A similar relationship between the concentrations of PQ (10^{-6} and 10^{-5} M) was observed when carbachol (3×10^{-4} M) was given. From these results, it is suggested that a neuropharmacological mechanism may be involved in the action of PQ.

Pax et al.[13] showed in *S. mansoni* that PQ decreased the influx of K^+, but stimulated the influx of Na^+ and Ca^{2+}. They then suggested that the spastic paralysis in the motility of the worm may be related to depolarization of the cells, and be elicited through inhibiting Na^+, K^+-ATPase-like functions of the worm. Coles[14] also suggested in *S. mansoni* that PQ opens pores in the membrane and permits a rapid influx of Ca^{2+}, either directly or indirectly through an effect on the influx of Na^+. In this study, ouabain, a well-known inhibitor of Na^+, K^+-ATPase, showed similar effects on many preparations such as *D. caninum, P. westermani, T. vulpis* and the isolated frog rectus. The figure (E and F) shows effects of PQ, ouabain and eserine on the motility of *P. westermani.* Ouabain (3×10^{-5} M) caused spastic paralysis similar to that caused by PQ (10^{-7} M), which was restored by the addition of eserine (2×10^{-5} M).

There have been many reports regarding the relationship between Na^+, K^+-ATPase and energy metabolism[22,23] including glucose uptake and also cation transport[22,23], including nervous conductance. There have been also many reports regarding the involvement of Na^+, K^+-ATPase as well as Mg^{2+}-ATPase in the uptake, storage and release of neurotransmitters[23–25]. Thus, biochemical and neuropharmacological changes due to PQ may be elicited secondarily through the action of this compounds on Na^+, K^+-ATPase-like functions in worms. Additionally, PQ is reported to make worms more susceptible to proteolytic enzymes[9], and also to cause morphological changes in mitochondria[12]. These changes may also be attributed to the above-mentioned mechanism. We are now investigating the relationships between the inhibition of ATPases and other changes.

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Depth perception by means of ambient sounds in a small mammal[1]

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Summary. Golden hamsters placed on a jumping stand from which they can descend onto a shallow or deep landing platform prefer to descend on to the shallow platform, even when tested under IR-light without tactile cues. This preference disappears for subjects with plugged ears. The simultaneous recording of the animal's behaviour and possible emission of ultrasound as well as experiments in which the external acoustical conditions or the sound-reflecting properties of the jumping apparatus were altered suggest that the animals use certain parameters of the ambient sound field for depth perception.

Depth perception in mammals has mainly been studied with respect to vision (for a summary of the literature, see Thinus-Blanc[2]). The perception of space, however, may involve sensory modalities which vary according to the animal's sensory equipment and way of life in a given environment. Furthermore, a given species may use different categories of external cues in different test situations.

Golden hamsters *(Mesocricetus auratus W.)* are active at night and probably spend most of their time underground, like other burrow-dwelling rodents. Extensive experiments with a visual cliff have shown that these animals can use visual criteria for depth perception, but that they are more influenced by tactile stimuli if both categories of cues are presented in a conflicting way[3]. Similarly, the behavior of hamsters tested on a real cliff, where the animal cannot use tactile cues, reveals that the estimate of depth is more accurate under white light, yet is by no means abolished in complete darkness[4]. Thus, depth perception seems to occur in this species when both visual and tactile stimuli are eliminated. The aim of this research was, therefore, to examine the role of auditory cues in the above-mentioned situation. If these cues should prove to provide the animals with the relevant information, further experiments should establish a) whether the subjects themselves produce sounds which are reflected by nearby objects (active echolocation), or b) whether they perceive certain features of their acoustical environment which translate the interaction between external sound waves and objects. These features may correspond to patterns of sound reflection, absorption and diffraction.

In most experiments, golden hamsters of both sexes, aged 3–20 months, were tested on the jumping stand represented in figure 1a, which was located in a room A, on the 1st floor of a city building. Each subject was placed by an experimenter on the center of the start platform, its head facing one of the 2 plexiglas walls. To place the animal correctly, the experimenter very briefly used a weak electric torch. The animal was then able to leave the start platform by choosing one of the 2 landing platforms, located at a distance of 20 cm and 105 cm from the start platform. The cliff, 20 cm deep on the shallow side of the apparatus, did not allow the subjects to obtain tactile information through the vibrissae during frequent head dippings which occured at the edge of the start platform. To exclude visual information, the animals were always video-filmed under IR-light; the IR-projector containted a halogene tube (supplied by $\leq$ 100 W) and a Shottfilter which transmitted 50% light at 850 nm, and 10^{-3}% light at 780 nm. According to previous behavioral and electrophysiological experiments, the hamster's visual responsiveness to red and near-IR wavelengths stops at 740 nm[5]. Precautions were taken to eliminate olfactory cues which might have been left on the apparatus by previously tested subjects: after each experiment, the start- and landing platforms were thoroughly cleaned with alcohol and water. To neutralize possible side preferences, the heights of the 2 landing platforms were exchanged after every 2nd experiment. Animals which did not leave the start platform after 20 min, which left the platform through accidentally falling off, or before they had explored both sides of the cliff with head dippings, were excluded. 'Naive' animals, which had never been tested on the jumping stand, were given the opportunity to explore the latter approximately 5 h before the test, with both landing platforms being raised to a distance of 5 cm below the start platform.

The animals were observed on a video-monitor located in an adjacent room. Their behaviour was recorded in terms of 1. the choice of descending either on the near or distant landing platform, 2. the latency of this choice, and 3. the number of head dippings (animal holding itself with forepaws at the edge of the start platform and lowering its head beneath the level of the platform either very briefly or with superimposed horizontal exploratory movements) on either side of the start platform prior to leaving it.

In previous experiments, the subjects increased the latency with which they left a circular jumping platform as a function of the latter's height above floor level[4]. It was, therefore, to be expected that in the choice situation of the present experiment, the hamsters would prefer to descend on the shallow, rather than on the deep side of the jumping apparatus. Figure 2a shows that this was indeed the case, both for naive subjects tested for the first time and for ex-