samples were treated with 0.2*N* NaOH for 10 minutes at room temperature, the direct-reacting bilirubin was converted to indirect-reacting bilirubin, and the capacity to form hydroxamate was lost. The data presented in Table 1 show a fairly constant proportionality between the decrease in hydroxamate formation and the amount of direct-reacting bilirubin converted to the indirect-reacting form.

The direct-reacting bilirubin in urine was converted to the stable azopigment B (*3*) by coupling with diazotized sulfanilic acid. The product was extracted with *n*-butanol, purified by ascending chromatography on washed Whatman No. 3 paper, with water-saturated *n*-butanol, acetic acid (4/1) as the solvent, and eluted with absolute methanol. Azopigment B can be converted to azopigment A, with the liberation of glucuronic acid, either by dilute alkali (0.2*N* NaOH, 10 minutes, room temperature), by acid (1*N* H_2SO_4, 80 minutes, 100°C), or by the action of bacterial β-glucuronidase (Sigma, 20 mg/ml, *p*H 6, 38°C, 18 hours) (*1, 3*). With ascending chromatography on Whatman No. 1 paper and with water-saturated *n*-butanol, acetic acid (4/1) as the solvent, azo-pigments B and A migrate with R_f values of 0.36 and 0.55, respectively.

Treatment of azopigment B with an excess of hydroxylamine (*p*H 7, room temperature, 30 minutes) yields a new pigment, which migrates as a single spot with an R_f value of 0.44. This product, assumed to be the hydroxamate of azopigment A, has an absorption spectrum at *p*H 6.0 identical with that of azopigment B between 330 and 600 mμ, with maximal absorption at 525 mμ. In contrast to azopigment B, the hydroxamate is free of hexuronic acid, estimated by the carbazole method (*6*), and is unchanged by dilute alkali or bacterial β-glucuronidase, as judged by paper chromatography. However, on treatment with 1*N* H_2SO_4 for 80 minutes at 100°C, the hydroxamate is converted to a pigment with an R_f value identical to that of azopigment A.

Table 1. Effects of mild alkaline hydrolysis on the van den Bergh reaction and on hydroxamate formation in the urine of patients with obstructive jaundice. The van den Bergh reaction was performed according to the method of Malloy and Evelyn (*7*), and hydroxamate was estimated as has been previously described (*4*); both are expressed in units of optical density.

Patient	Δ van den Bergh		Δ Hydroxamate
	Direct	Indirect	
D	−190	+150	−084
S	−616	+464	−226
N	−920	+696	−367

These observations indicate that bilirubin diglucuronide, and the azopigment B derived from it, react with hydroxylamine to form the corresponding hydroxamic acids. This reaction, specific for *acyl* glucuronides, provides direct confirmation that bilirubin is conjugated with glucuronic acid through its carboxyl groups.

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Absence of Chitin in Epicuticle of Some Indian Scorpions

Epicuticle of arthropods is defined by Richards (*1*) as a characteristically nonchitinous layer. Krishnan, Ramachandran, and Santanam (*2*), on the other hand, demonstrated the presence of chitin in the epicuticle of a scorpion *Palamneus swammerdami* (*3*). It was therefore decided to test the epicuticle of other scorpions for chitin.

The scorpions *Palamnaeus bengalensis* and *Buthus tamulus gangeticus,* commonly found at Lucknow, were used for the study. When pieces of cleaned cuticle of freshly killed scorpions were subjected to the chitosan test, the epicuticle gave a negative reaction for chitin. Some pieces of cuticle which had been previously treated with alkali during the chitosan test were placed in 3 percent acetic acid overnight, but the epicuticle did not show dissolution. This would suggest further that the epicuticle is nonchitinous.

Krishnan *et al.* (*2*) had suggested that the epicuticle of *P. swammerdami,* unless previously treated with chlorated nitric acid, would not respond to the chitosan test. Even repetition of Krishnan's method in the cuticle of *P. bengalensis* and *B. tamulus gangeticus* did not give, with 1 percent sulfuric acid, any violet coloration characteristic of chitosan in the epicuticle. This would indicate that chitin is absent from the epicuticle of the scorpions *P. bengalensis* and *B. tamulus gangeticus.*

X-ray diffraction studies were also carried out in order to detect the presence, if any, of chitin in the epicuticle of these scorpions. Pieces of cuticle of the two scorpions under experiment were treated with several changes of cold, freshly prepared concentrated chlorated nitric acid till the epicuticle was completely freed of the underlying exocuticular material. Pieces of epicuticle thus obtained were thoroughly washed in running water and then in distilled water. For the x-ray diffraction experiment, 12 pieces were mounted, one over the other, on a small hole on a glass slide and left in a desiccator overnight. The pieces of epicuticle stuck together and to the slide during the drying process; after this the x-ray diffraction photographs were taken. Cu-K-alpha radiations were used, and the object was kept perpendicular to the beam and at a distance of 5 cm from the photographic plate.

Figures 1 and 2 show the diffraction patterns of the epicuticle of *P. bengalensis* and *B. tamulus gangeticus,* respectively. Prominent d-spacings in the epicuticle of *P. bengalensis* and *B. tamulus gangeticus* occur at 5.302 A and 3.901 A (faint) and at 3.007 A, 3.941 A, and 5.219 A, respectively.

Krishnan *et al.* (*2*) have suggested the presence of chitin in the epicuticle of *P. swammerdami* on a comparison of the d-spacings 3.37 A, 4.1 A, and 9.1 A obtained in the epicuticle after removal of the outer paraffin layer present in their scorpion, and of the d-spacings 2.22 A, 2.44 A, 3.7 A, and 4.14 A obtained in the entire epicuticle, with the d-spacings 3.3 A, 4.55 A, 5.0 A, and 9.9 A obtained in the purified chitin of *P. swammerdami.* The d-spacings obtained in the epicuticle of the scorpions *P. bengalensis* and *B. tamulus gangeticus* are different from all the d-spacings obtained by Krishnan in *P. swammerdami.* Furthermore, the d-spacings of the epicuticle of *P. bengalensis* and *B. tamulus gangeticus* do not tally with the d-spacings of chitin from other arthropods.

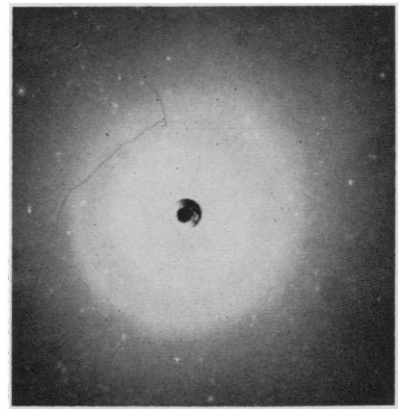

Fig. 1. X-ray diffraction photograph of the epicuticle of *P. bengalensis.*

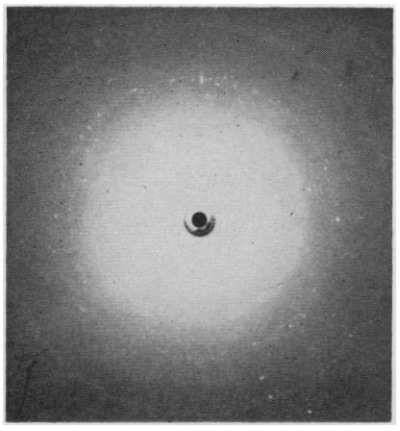

Fig. 2. X-ray diffraction photograph of the epicuticle of *B. tamulus gangeticus*.

It is evident that the epicuticle of the scorpions *P. bengalensis* and *B. tamulus gangeticus* is nonchitinous.

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Withdrawal of Positive Reinforcement as Punishment

Many experiments have shown that an organism's behavior can be maintained by the use of positive reinforcement (reward). At the same time, the withdrawal of the situation in which the reinforcement occurs has been described as an aversive event (*1*) and is called a "time out." This experiment (*2*) shows some of the aversive properties of a time out from positive reinforcement in chimpanzees. The procedure here is very similar to one used by Azrin in which electric shock is the aversive event and pigeons are the subjects (*3*). In such cases, light or sound (pre-aversive stimulus) preceding the aversive event disrupts the animals' usual performance.

In this experiment the aversive event (time out from positive reinforcement) was established as follows. The chimpanzees pressed a telephone key, which occasionally produced food. An overhead light in the experimental chamber was periodically turned off, and at the same time the food magazine was disconnected from the key. The animals soon stopped pressing the key in the absence of the overhead light. Thereafter, the experiment could be interrupted conveniently for any period of time by turning off the overhead light.

A red lamp, called the pre-time-out or pre-aversive stimulus, was then installed next to the key. The light appeared every 15 minutes for 160 to 180 seconds, depending on the animals' performance. If an animal pressed the key during the last 20 seconds of the pre-time-out period, a 60-minute time out followed; but if it did not press the key during the final 20 seconds, the red light terminated, and no time out could occur until 15 minutes later, when the pre-time-out stimulus reappeared.

Key presses were reinforced on a variable-interval schedule in which the first key press after varying periods of time was reinforced. The mean interval between reinforcements was 6 minutes, with a range between zero (successive responses) and 12 minutes.

Figure 1 contains an entire daily record, 6 hours long, representing the stable performance after several hundreds of hours of exposure to the experimental procedure. Responses are cumulated against time, and the diagonal strokes on the curve indicate the start and end of the pre-time-out stimulus. The performance was recorded continuously; however, to facilitate inspection of the performance during the pre-time-out stimulus, the curve was broken into 15-minute segments with the pre-time-out period in the middle. The grid in the lower right part of the figure gives the coordinates, the scale of the record, and several reference slopes in responses per second. The reinforcements are not shown on the curve.

The resulting performance with the time out as the aversive event closely parallels the data reported by Azrin. The aversive properties of the time out appeared as suppression of the key-pressing during the pre-time-out stimulus. The degree of suppression ranged from the performance in the fourth segment of the second column, where suppression is complete, to the performance in the preceding segment, where the chimpanzee stops pressing the key only during the last 35 seconds. In most cases the chimpanzees pressed the key during the early part of the pre-time-out stimulus at the rate of responding that generally prevailed elsewhere, but they stopped abruptly some time before the final 20 seconds, when a response would produce a time out. Time outs occurred in approximately 3 percent of the pre-time-out stimuli. In the session represented in Fig. 1 a time out occurred at the arrow.

The suppression of the base-line behavior by the stimulus preceding the time out from the variable-interval schedule of reinforcement establishes the time out as an aversive event having properties similar to those of electric shock. The time out as an aversive event can be extrapolated to most aversive control in human behavior, where noxious stimuli such as corporal punishment or electric shock are rarely used. On the other hand, positive reinforcement is frequently discontinued, as in fines, disapproval, or incarceration. The important feature in such types of control is that an individual is punished by the withdrawal of the reinforcements for significant segments of his behavioral repertoire. Such withdrawal is similar to the time out of the present experiment.

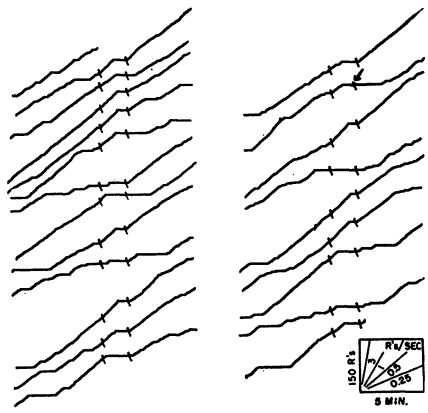

Fig. 1. Complete daily session showing suppression of key-pressing during the pre-time-out stimulus.

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Gamma Globulin Factors Protective against Infections from Pseudomonas and Other Organisms

In mice rendered susceptible to *Pseudomonas aeruginosa* infections by pretreatment with cortisone or by extensive thermal trauma, human gamma globulin (GG) administered after the infection was shown to be highly active in preventing death (*1*). The nature of