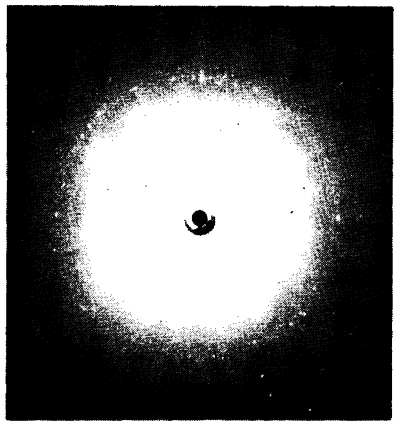

Fig. 2. X-ray diffraction photograph of the epicuticle of *B. tamulus gangeticus*.

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

M. B. LAL
S. C. SHRIVASTAVA
Zoological Laboratory,
University of Lucknow, India

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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.

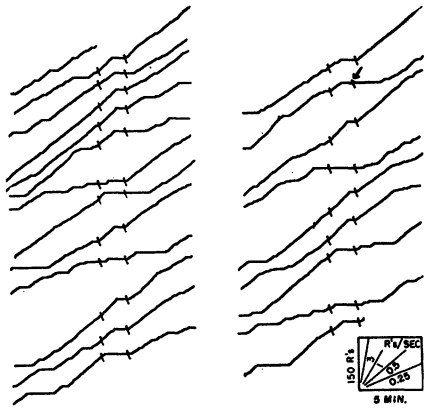

Fig. 1. Complete daily session showing suppression of key-pressing during the pre-time-out stimulus.

C. B. FERSTER*
Yerkes Laboratory of Primate Biology, Orange Park, Florida

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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

this protective action has not been clarified, although evidence was presented suggesting that the properdin system was not responsible (*1*).

The present report (*2*) demonstrates the protective action of gamma globulin against a variety of other organisms, Gram-positive as well as Gram-negative; furthermore, an assay of plasma, based on protection of mice against *Ps. aeruginosa,* is described, and the titers in some laboratory animals are reported.

In this study, the organisms were injected intraperitoneally, suspended in 0.4 to 0.5 ml of mucin, as a means of enhancing the infection (*3*). Mucin obviated the use of cortisone and produced infections that were more rapidly fatal (12 to 36 hours). Results with this procedure were found to be more reproducible, particularly in the assay of titer of plasma preparations by mouse protection tests, described in the following paragraph. From one to ten lethal doses of organisms were used, and the gamma globulin or plasma was administered intraperitoneally, 3 to 4 hours prior to the infection, unless otherwise stated. Preparation of the organisms was similar to that previously described (*1*).

Table 1. Protective action of human gamma globulin against various infections in mice. Centrifuged organisms suspended in 0.9 percent NaCl to optical density of 0.45 to 0.50 at 650 mμ, before dilution in 5 percent gastric mucin (hog); 0.4 to 0.5 ml of suspension inoculated intraperitoneally; 0.1 ml (16 mg) of gamma globulin given intraperitoneally 3 to 4 hours before inoculation.

Organism, type or strain	Organism diln. in mucin	No. of mice in each group	72-hr mortality (%)	
			Untreated	GG-treated
Ps. aeruginosa				
Strain 180	10^{-3}	30	90	13
	5×10^{-3}	20	95	20†
	5×10^{-3}	10	95	40‡
Strain 181	10^{-3}	10	90	40
Parker	10^{-3}	10	90	10
G-140	10^{-2}	10	90	40
E. coli				
Rowley 0111:B4:H12	10^{-5}	20	95	100
	10^{-6}	10	100	90
Stokes 0111:B4	10^{-3}	20	60	60
08:K42(A)	10^{-2}	10	90	90
	10^{-3}	20	95	65
09:K29(A)	10^{-2}	10	100	70
6641(L)	2×10^{-1}	10	80	30
ATCC 9637	10^{-1}	10	80	0
02:K1(L)	10^{-5}	10	100	100
010 K5(L)	10^{-3}	10	100	100
	10^{-4}	10	80	90
	10^{-5}	10	90	70
Proteus				
mirabilis	10^{-5}	10	100	10
mirabilis, Kf20	10^{-3}	10	100	0
vulgaris, Kf56	10^{-3}	10	40	60
morgani, C90	10^{-1}	10	70	50
rettgeri, 1165	10^{-1}	10	80	50
Klebsiella				
Kl. pneumoniae K-2	10^{-2}	10	80	100
Bact. aerogenes K-10	10^{-2}	10	70	0
Bact. aerogenes 1033	10^{-1}	10	70	0
Bact. aerogenes 8329 (ATCC)	10^{-4}	10	90	20
*St. aureus**				
Wood, ATCC 10832	10^{-1}	20	100	20
Georgio	10^{-1}	10	100	30
713 A	10^{-1}	10	100	20
Smith	10^{-5}	20	100	5
	10^{-5}	10	90	0†

* Cultures diluted in mucin without centrifugation.
† Gamma globulin given intravenously immediately after inoculation.
‡ Gamma Globulin given intramuscularly 5 hours before inoculation.

Protection with human gamma globulin was demonstrated against several strains of *Ps. aeruginosa, Escherichia coli, Proteus, Klebsiella,* and *Staphylococcus aureus* (Table 1). Marked protection was found against four strains of *Micrococcus pyogenes* var. *aureus,* with gamma globulin given intraperitoneally 24 or 4 hours before the infection or intravenously shortly after the infection. Similar protection was shown against *Ps. aeruginosa,* except that less effect was present with gamma globulin administered intraveneously, post infection; however, good protection was obtained under these conditions with a smaller inoculum. Activity against *E. coli* was weak, with the exception of two strains of low mouse virulence. Protection was shown against three strains *of Bacterium aerogenes* but not against one strain of *Klebsiella pneumoniae.* Further work is required to determine the relation of protective titer to pathogenicity or antigenic types of bacteria. Since human gamma globulin prepared for the American Red Cross represents samples pooled from 20,000 individuals, the activity may be taken as representing the mean of large sectors of the population.

The assay of protective titer of the plasma of laboratory animals was similar to the experiments just described; protective action of citrated plasma was tested in mice inoculated intraperitoneally with *Ps. aeruginosa,* strain 180, in mucin. To increase the sensitivity of the assay system, advantage was taken of the synergistic effect obtained when certain antibiotics are combined with gamma globulin or plasma (*4*). Thus, a single dose of oxytetracycline (8 to 10 mg/kg) given subcutaneously, immediately after the infection, was essentially without effect when given alone, but it enhanced the activity of gamma globulin or plasma several-fold. The use of antibiotics was advantageous with plasma of low titer, such as in the mouse, and it was employed routinely in all cases, so that comparative data for the various animals could be obtained.

The results (Table 2) indicate an order of potency of plasma from adult animals as follows: human being, dog, rat, rabbit, guinea pig, and mouse. These values must be accepted as only tentative, since the titer may be influenced by the strain, age, and past history of the animal; preliminary experiments indicate weaker titers in young animals. With the use of oxytetracycline, the potentiation of human plasma appears greater than that of human gamma globulin, although simultaneous com-

Table 2. Protective action of plasma of various laboratory animals against *Pseudomonas* infection in mice when combined with antibiotic therapy. Gamma globulin or plasma given intraperitoneally 3 to 4 hours before inoculation; oxytetracycline, subcutaneously, immediately after inoculation. Human plasma was a lyophilized preparation of commercial manufacture. *Ps. aeruginosa,* 10^{-3} dilution of organisms in mucin; 0.4 ml intraperitoneally. Since all plasmas were collected in citrate solution, a 30-percent dilution factor should be applied, except for the lyophilized human plasma.

Therapy	No. of mice	72-hr mortality (%)
No treatment	80	96
Oxytetracycline (8 to 10 mg/kg)	80	93
Human GG alone, 8 mg	29	33
Human GG + oxytetracycline		
0.5 mg	50	54
1.5 mg	50	42
5.0 mg	20	10
Human plasma alone, 0.1 ml	10	70
Human plasma + oxytetracycline		
0.01 ml	20	35
0.03 ml	30	13
0.10 ml	20	0
Dog plasma + oxytetracycline		
0.03 ml	10	80
0.10 ml	20	20
0.30 ml	10	10
Rat plasma + oxytetracycline		
0.1 ml	20	60
0.3 ml	20	35
1.0 ml	10	20
Rabbit plasma + oxytetracycline		
0.1 ml	10	80
0.3 ml	10	60
Guineapig plasma + oxytetracycline		
0.3 ml	30	73
1.0 ml	30	53
Mouse serum + oxytetracycline		
0.3 ml	10	80*
1.0 ml	10	80*

* Serum-treated mice in these experiments died at a slower rate than did untreated mice, indicating the presence of a small amount of protective activity in mouse serum.

parisons of gamma globulin prepared from the same plasma are needed to establish this.

Gaines and Landy (*5*), employing a hemagglutinin technique, demonstrated antibodies in normal human serums against a *Pseudomonas* antigen; higher titers were present in older age groups. Various investigators have demonstrated small amounts of *Staphylococcus* antitoxin in the serums of man and animals, and the antibacterial properties of normal serum have been widely investigated for many years (*3*). The role of these *in vitro* properties of serum in its protective action against lethal infections in animals remains to be established. However, Cameron (*6*) has demonstrated protective action from gamma globulin derived from several domestic animals against experimental infections with *Pasteurella multocida, Salmonella choleraesuis* and *Brucella suis.*

R. Carl Millican
J. Rust
S. M. Rosenthal
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

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Interrelationship between Certain Bacteria and the Rumen Ciliate Dasytricha ruminantium

The cattle and sheep ciliates of the genera *Isotricha* and *Dasytricha* have been shown to possess considerable fermentation ability (*1, 2*). Soluble carbohydrates are rapidly utilized by the protozoans, with the production of acids and gas and with concomitant deposition of food reserve amylopectin. The nature of the nitrogen source for the holotrich ciliates has been elusive (*3*). The holotrich protozoans do not easily lend themselves to microscopic observation of bacterial feeding. In well-fed ciliates the entire organism is loaded with reserve food granules, making it difficult to detect either the presence of bacteria within the ciliates or bacteria actually being ingested by the protozoans.

Growth experiments with the holotrich protozoan *Isotricha intestinalis* have been reported (*2*). The ciliates grew during a short period in an anaerobic medium containing ground alfalfa, wheat, and untreated rumen fluid in inorganic saline. Counts of the protozoans during this time indicated division every 48 hours. The smaller, more abundant holotrich found in the rumen, *Dasytricha ruminantium,* did not survive in these experiments, indicating a difference in cultural requirements. It was of interest to find what these differences were and also what factors would stimulate growth of *D. ruminantium* in laboratory cultures.

Starvation of *D. ruminantium* for 48 to 72 hours decreased the number of amylopectin reserve granules and facilitated the search for bacterial ingestion by the protozoans. The starved ciliates were placed on a slide with a small amount of 0.5 percent saline and a mixture of bacteria obtained from fresh rumen fluid by centrifugation. A cover glass, ringed with Vaseline, excluded air from the entire suspension. With this arrangement the protozoans remained active and could be observed for several hours, with the use of a phase microscope. *Dasytricha ruminantium* selected and ingested small cocci, 0.5 to 0.8 μ in diameter, from the mixture. The bacteria agglutinated at the mouth located at the posterior of the organism and were gradually ingested. Occasionally, a ciliate was observed which swam about trailing many agglutinated bacteria. Small rod-shaped bacteria, 1.0 × 1.5 μ in size, were seen also within food vacuoles in the ciliates. In several of the experiments, exposure of the bacterial suspension to triphenyltetrazolium chloride allowed more distinct observation of bacterial ingestion by the protozoans. Bacteria which stained red as a result of the deposition of the insoluble formazan were seen localized near the mouth and in the interior of the ciliate in food vacuoles.

Several bacterial strains were isolated from rumen contents in order to test their growth-promoting effect on *Dasytricha.* Following the initial isolation on a starch-alfalfa extract agar, the bacteria were grown in liquid medium of the same type and used in the ciliate cultures. Small vessels with a flat upper surface and an opening fitted with a rubber stopper allowed observation of the protozoans without exposure to air. With the aid of a dissecting microscope, the response and fate of the ciliates in each experiment could be followed easily. With cooling of the cultures from 39°C to room temperature, most of the protozoans decreased their swimming activity and settled to the bottom of the vessel. The stale supernate was then removed with a capillary pipette, while anaerobic conditions were preserved by passing carbon dioxide into the vessel opening. Fresh 0.5 percent buffered saline containing substrate and bacteria was added every 24 hours. Streptomycin and penicillin, plus washing techniques, were used to free *Dasytricha* of most of the associated rumen bacteria.

Dasytricha ruminantium was cultured for 2 weeks in the presence of two species of rumen bacteria and the following components, given in percentage: 0.05 ground alfalfa, 0.005 cornstarch, 0.5 autoclaved rumen fluid, 0.005 glucose, and a mixture of ten vitamins. Cysteine hydrochloride, 0.01 percent, was added to insure reduced conditions. Numerous dividing ciliates were observed each day. Parallel control cultures which omitted the bacteria were dead at 96 hours. The bacterial strains which stimulated growth of *Dasytricha* were a small coccus, 0.8 μ in diameter, having a large mucoid colony, and a rod 1.0 × 1.5 μ in size, possessing a filamentous type colony. The larger holotrich ciliates of the genus *Isotricha* were also observed feeding on rod-shaped bacteria (*4*). It is concluded that the holotrich rumen ciliates derive at least some of their nitrogenous requirements from the ingestion of associated bacteria.

Jose Gutierrez
Animal Husbandry Research Division, Agricultural Research Center, USDA, Beltsville, Maryland
R. E. Hungate
Department of Bacteriology, University of California, Davis

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8 July 1957

Rapid Chemical Changes in Reconstituted Dry Milk

Dry milk is usually reconstituted into an aqueous system before use as a food, and many of the laboratory analyses of dry milk involve reconstitution with water as a step in sample preparation. Recent study of certain chemical properties of reconstituted nonfat dry milk indicated a variability in analytic results which was correlated with the age of the reconstituted samples. The purpose of this report is to illustrate some rapid chemical changes which may occur in freshly reconstituted dry milk in order to bring the phenomenon of change to the attention of those interested in the