virus inactivation by the digested and undigested antibodies were performed according to the procedure of Lafferty (*5*).

As shown in Fig. 2, no definite dissociation of antibody fragments from the virus was observed when the reaction mixture was diluted in a neutral medium. Furthermore, under the same conditions there was no evidence of dissociation when combined ^{32}P-labeled virus and monovalent antibody fragments were subjected to chromatography (*11*).

Thus, the neutralizing capacities of papain-digested antibodies against three different types of virus appear to differ. Type 5 adenovirus could not be neutralized by univalent (adenovirus) 3.5*S* antibody fragments. Fragments derived from rabbit antibody against influenza A virus showed neutralizing ability with influenza A virus, but the complex dissociated almost completely on dilution in a neutral saline medium. Our experiments demonstrate the neutralizing capacity and the irreversible binding of the antibody fragments to poliovirus particles.

ARNOLD VOGT, RUDOLF KOPP
GÜNTER MAASS, LEO REICH
Hygiene-Institut der Universität, Freiburg i.Br., Germany

References and Notes

1. R. R. Porter, *Biochem. J.* **73**, 119 (1959).
2. A. Taranta and E. C. Franklin, *Science* **134**, 1981 (1961).
3. Z. Ovary and F. Karush, *J. Immunol.* **86**, 146 (1961).
4. J. H. Baxter and P. A. Small, Jr., *Science* **140**, 1306 (1963).
5. K. J. Lafferty, *Virology* **21**, 76 (1963).
6. L. Kjellén, *Arch. Virusforsch.* **14**, 189 (1963).
7. Worthington Biochemical Corp.
8. J. S. Youngner, *Proc. Soc. Exptl. Biol. Med.* **85**, 202 (1954).
9. L. J. Reed and M. Muench, *Am. J. Hyg.* **27**, 493 (1938).
10. Nutritional Biochemicals Corp.
11. R. Thomssen, in preparation.
12. Supported by a grant from Deutsche Forschungsgemeinschaft.

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Bee Venom Tolerance in White Mice in Relation to Diet

Abstract. *In white mice the consumption of a high protein diet either just before, or over a period of 3 days prior to, the injection of venom from the honey bee* Apis mellifera *L. markedly increases the number of deaths. Conversely, a period of starvation or a nonprotein diet fed to white mice for 3 days prior to the injection of bee venom significantly reduces the number of deaths.*

A review of the literature indicated that no studies have been made on the relation of the composition of diet and time of feeding to the tolerance or detoxification of insect poisons in mammals, or both. Our experiments were undertaken to establish these relations.

The venom used in our experiments was collected in July 1963 by the method of Benton, Morse, and Stewart (*1*). The venom was a composite sample collected over a period of 1 week from 25 hives of bees. A stock solution was prepared by dissolving 200 mg of the pooled venom as completely as possible in 50 ml of 0.85 percent saline solution. The solution was filtered, sterilized in an autoclave, and frozen at −15°C in 10-ml serological vials. The number of micrograms of solid per milliliter was determined by drying and weighing a known volume of the venom. The stock solution contained 5 μg of venom per milliliter.

Female white mice (23 to 28 g) of the Swiss Webster strain were kept in an environmental growth chamber in 12 hours of light and 12 hours of darkness at 23.5° ± 0.5°C and a relative humidity of 65 percent ±1.5 percent. At 8:00 A.M. and 5:00 P.M. they were offered 2.5 g of rodent food per mouse. When special diets were substituted for the rodent food, the mice were offered an equivalent amount of food on the same schedule. The diets, obtained from Nutritional Biochemicals Corporation (*3*), were placed in contamination-free feeders to which the mice had access. Distilled water was available to the mice at all times.

The mice (except where otherwise stated) were injected by the intraperitoneal route as described by Campbell *et al.* (*2*). Six-tenths centimeter, 27-gauge needles with 0.25-ml syringes were used. All mice were weighed to the nearest 0.1 g prior to injection (10 to 20 minutes). The volume of bee venom administered was adjusted for each mouse on the basis of this body weight.

Fifteen mice were used for each determination, and each experiment was replicated three times. In experiments where the mice were injected immediately after feeding, 0.3 g was deducted from the weight of each mouse to compensate for the food which it had eaten, since this was found to be the maximum amount of food consumed by a series of mice feeding in a 25- to 30-minute period. All mice were injected with 0.21 μg of bee venom per gram of body weight. This is the concentration required to kill 5 mice out of a total of 15 mice in a 24-hour period under normal conditions (normal diet and no starvation period prior to feeding). All mice were injected at 3:00 P.M. to compensate for possible fluctuations due to daily rhythm.

Table 1. The tolerance of white mice to bee venom related to the time of feeding and diet. A, Starvation 18.5 hours prior to feeding; B, food available at all times; C, starvation 6 hours prior to injection. Three replications in each schedule.

Diet	Feeding schedule	Number dead after 24 hours (three replications)		
Peanut butter	A	11	10	11
Normal (protein, 27%)	B	5	5	5
Normal (protein, 27%)	C	1	0	2

Table 2. The relation of diet to the tolerance of white mice to bee venom. Fifteen mice were used in each experiment. The animals in group A were fed 20 minutes prior to injection after an 18-hour period of starvation, and those in group B were fed for 3 days prior to injection. There were three replications in each group. The number of deaths are recorded in the table.

Group A (No.)			Group B (No.)		
		High protein, 64%			
9	10	10	11	12	10
		High fat			
6	5	5			
		High carbohydrate			
5	4	5			
		No protein			
4	5	5	2	2	0
		Normal protein, 27%			
			5	5	4

There was a decrease in the number of deaths (Table 1) when the mice were starved for 6 hours prior to injection. Conversely the number of deaths rose sharply after a meal of peanut butter. These data indicated that the time of feeding and possibly the diet played an important role in the toxicity of bee venom to white mice and formed the basis for further experimentation.

To eliminate the possibility that an intake of any food might cause the same effect, mice which had been maintained on a normal protein diet were starved for 18.5 hours and then allowed to feed for 20 to 30 minutes on diets which varied in protein, carbohydrate, and fat content (*3*). The mice were injected within a 15-minute period after a feeding. The data are shown in Table 2.

The number of mice killed by venom when fed a high-protein diet was twice as great as when they were fed the other diets; therefore, protein intake directly or indirectly affects the tolerance of mice to bee venom. In the aforementioned experiment white mice were fed diets, which varied in the percentage of protein, for 3 days (Table 2). The mice were injected at 3:00 P.M. with no starvation period preceding the administration of the bee venom. In this experiment not only was there a substantial increase in the number of deaths among the mice fed a high-protein diet, but the number of deaths was also reduced by two-thirds among those mice fed on a diet which contained no protein.

Mice that were 14 weeks old were twice as susceptible to bee venom as 8-week-old mice when all were kept on a normal daily routine with a normal protein diet. Ten mice out of 15 died in the 14-week-old group whereas only an average of 5 mice out of 15 in the 8-week-old group died when both groups were injected with a dose calculated to kill 5 out of 15. It also was determined that mice injected subcutaneously with 75 μg of venom per gram of body weight did not die. This is ten times the amount required to produce 100 percent killing when injected into the peritoneal cavity. In fact, the animals completely recovered in 6 to 12 hours.

ALLEN W. BENTON
ROGER A. MORSE
ALBERT F. GUNNISON
Department of Entomology,
Cornell University, Ithaca, New York

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References and Notes

1. A. W. Benton, R. A. Morse, J. D. Stewart, *Science* **142**, 228 (1963).
2. D. H. Campbell *et al.*, *Methods in Immunology* (Benjamin, New York, 1963), pp. 9–11.
3. The diets were obtained from the Nutritional Biochemicals Corporation, Cleveland 28, Ohio. They are catalogued as High Carbohydrate Diet, High Fat Test Diet, High Protein Diet, Normal Protein Test Diet, and "Protein Free" Diet. The peanut butter was a nonhomogenized commercial brand.
4. Supported by NSF research grant GB 327, NIH research grant GM 10897-04, and USPHS predoctoral fellowship 1-FI-GM-20, 129-01.

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Sulfate Transport in Human Red Cells: Inhibition by Some Uncouplers of Oxidative Phosphorylation

Abstract. *Release of inorganic sulfate from human erythrocytes is depressed in the presence of 2,4-dinitrophenol (5×10^{-4}M) or dicumarol (5×10^{-4}M). This effect cannot be readily attributed to uncoupling of phosphorylation from respiration, since the study was conducted with cells that metabolize principally by anaerobic means and since the effect was not influenced by iodoacetic acid. A more reasonable explanation may be that permeability of the erythrocyte membrane to anions may be reduced by these agents.*

Uncouplers of oxidative phosphorylation, such as 2,4-dinitrophenol (DNP), are often used in studies of cell physiology to evaluate the relation between metabolism and function. The best known action of these agents is that they prevent formation of adenosine triphosphate (ATP) in mitochondria by dissociating reactions concerned with phosphorylation from those concerned with electron transfer (*1*). A change in function induced by uncoupling agents in whole, intact cells, however, cannot be ascribed simply to depressed mitochondrial phosphorylation because these inhibitors could conceivably affect some other process in the more complex, intact cell system. Thus, DNP depresses the resting membrane potential in certain excitable tissues (*2, 3*), but this change cannot be readily attributed to reduced synthesis of high-energy phosphate in the absence of more direct evidence, such as a reduction in concentration of ATP in the membrane. My report concerns an action of certain uncoupling agents on the human erythrocyte which cannot be readily explained by disruption of oxidative phosphorylation. This being the case, the question arises as to whether the effect of these inhibitors on other intact mammalian cells could also be caused by a less familiar action, such as the inhibition of ion transport observed in this investigation.

Blood collected in the usual mixture of acid, citrate, and dextrose (for blood storage) was centrifuged at 800*g* and 5°C for 5 minutes, and the buffy coat and plasma were removed. The cells were washed four times with a modified, calcium-free Ringer-Locke solution, volumes used being equal to that of the plasma removed. The salt solution was adjusted to *p*H 7.4 at 37.5°C with tris(hydroxymethyl)aminomethane buffer. After the separation and washing procedures, the cells were labeled by incubating them (*4*) for 1 to 2 hours at 37°C in calcium-containing Ringer-Locke medium in the presence of 0.5 μc of radioactive inorganic sulfate ($S^{35}O_4^{--}$) per milliliter of cells and 1m*M* sulfate. The labeled cells were then washed twice and resuspended in nonradioactive medium in order to study the release of sulfate ion.

Samples of the labeled blood suspension (3.5 ml) with a hematocrit value of 20 percent were placed in 25-ml erlenmeyer flasks to facilitate subsequent sampling, and the flasks were incubated. Portions (0.3 ml) of the blood suspension were removed after 10, 70, and 130 minutes, and the radioactivity was determined by plating the samples and the medium alone on concentrically ringed aluminum planchets; the samples were air-dried and counted with an end-window Geiger-Mueller counter. After correcting the counts for mass absorption, the results were expressed in terms of the ratio, medium $S^{35}O_4^{--}$: total $S^{35}O_4^{--}$.

The *p*H was monitored with a Beckman model G meter through external glass and reference electrodes that were placed in the incubator along with the blood samples. Decrease in *p*H was generally less than 0.05 *p*H units in 2 hours, and no significant difference was noted between control samples and samples containing an uncoupling agent. Hematocrit measurements (*5*) showed no detectable differences. Besides DNP (Eastman or Fisher), other inhibitors used were