of 5-hydroxytryptamine was not significantly changed by the administration of Tremorine.

Oxotremorine, a metabolite of Tremorine (*11*) was also investigated. When 200 μg/kg was injected intraperitoneally, the concentration of norepinephrine in the brain stem of the rat decreased, while the concentration of 5-hydroxytryptamine increased after 60 minutes, but the limited quantity we were able to obtain (*12*) did not allow further investigation.

These results are in partial agreement with recent clinical findings which suggest that there might be some biochemical malfunction in Parkinson's disease, especially with regard to the metabolism of catecholamines. They also appear to be relevant to findings in drug-induced extrapyramidal reactions (*13*). Our results with 5-hydroxytryptamine are not in agreement with those of Bernheimer, Birkmayer, and Hornykiewicz (*4*) who used brains from patients with Parkinson's disease, but appear to find support in the studies of Domer and Feldberg (*14*) who showed that intraventricular administration of 5-hydroxytryptamine enhanced, while catecholamines depressed, drug-induced tremors in the cat.

When Tremorine is administered to the adrenalectomized rat, the depletion of norepinephrine is increased, the concentration of 5-hydroxytryptamine is unaltered, and tremor is diminished; this suggests that Tremorine tremor, and perhaps parkinsonian tremor, involves both central and peripheral mechanisms, and that those in the periphery modulate those in the central nervous system by feedback.

The studies mentioned above also suggest why present-day pharmacotherapy of Parkinsonism is less than ideal. It is apparent that the basic disorder has not been clearly determined and that current attempts at correction offer only partial solutions of the problem. The exact role in motor function of the biogenic amines studied is still to be elucidated. It is significant that Tremorine, which gives rise to a condition in laboratory animals that simulates the Parkinson syndrome so remarkably, also reproduces acutely some of the biochemical features of this disease (see *15*).

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References and Notes

1. G. M. Everett, L. E. Blockus, I. M. Shepperd, *Science* **124**, 79 (1956).
2. A. H. Friedman and C. M. Smith, *J. Pharmacol. Exptl. Therap.* **135**, 62 (1962).
3. H. Ehringer and O. Hornykiewicz, *Klin. Wochschr.* **38**, 1236 (1960).
4. H. Bernheimer, W. Birkmayer, O. Hornykiewicz, **39**, 1056 (1961).
5. A. Barbeau, G. F. Murphy, T. L. Sourkes, *Science* **133**, 1706 (1961).
6. W. Birkmayer and O. Hornykiewicz, *Wien. Klin. Wochschr.* **73**, 787 (1961).
7. P. Shore and J. S. Olin, *J. Pharmacol. Exptl. Therap.* **122**, 295 (1958).
8. D. F. Bogdanski, A. Pletscher, B. B. Brodie, S. Udenfriend, *ibid.* **117**, 82 (1956).
9. J. A. R. Mead and K. F. Finger, *Biochem. Pharmacol.* **6**, 52 (1961).
10. Tremor activity was assessed by an arbitrary scale which evaluates the degree of tremor as well as the anatomical region involved. For example, mild tremor restricted to the ears is rated at 1+.
11. J. J. Kocis and R. M. Welch, *Pharmacologist* **2**, 87 (1960).
12. We gratefully acknowledge the gift of oxotremorine (as the picrolonate) from W. L. Haslett, University of California Medical Center, Los Angeles.
13. P. L. McGeer, *et al.*, *J. Am. Med. Assoc.* **177**, 101 (1956).
14. F. R. Domer and W. Feldberg, *Brit. J. Pharmacol.* **15**, 578 (1960).
15. A preliminary report of this study has appeared: A. H. Friedman, *Federation Proc.* **22**, 272 (1963). This study was supported by U.S. Public Health Service grant NB 03139-03.

12 July 1963

Antigenic Histamine Release from Passively Sensitized Human Leukocytes

Abstract. *With histamine release as an indicator, consistent passive sensitization of normal human leukocytes to specific antigen could be achieved with human serum, fresh or stored at −70°C, from allergic individuals. With certain sera, dilution slowed the rate of sensitization but increased eventual histamine release.*

When specific antigen is incubated with blood from a person with atopic allergy, histamine is released from the leukocytes (primarily basophiles) into the plasma (*1*). Since the same reaction occurs if antigen is added to washed leukocytes, free skin-sensitizing antibody has no apparent role in the reaction; apparently, the leukocytes have been sensitized optimally in vivo (*2*). Accordingly, the immunologic behavior of skin-sensitizing antibody in vitro can be studied only by passive sensitization of normal cells. However, the results of earlier attempts to accomplish such passive sensitization have been disappointing (*3*). In the one published report in which histamine release was the indicator, Middleton was successful in only three of ten experiments (*4*).

Table 1. Histamine released from normal cell suspensions after incubating 4 hours with diluted (50 percent) serum and 1 hour with Timothy antigen (.03 μg protein nitrogen per milliliter). The total cellular histamine was 85 μg/liter.

Cell samples	Incubation		Histamine (μg/liter)
	4 hours	1 hour	
1–2	Tyrode's	Antigen	5.9
3–4	A.S.*	Antigen	39.5
5–6	A.S.	No antigen	4.6
7–8	N.S.†	Antigen	5.1

* Allergic serum (pt. *Sh.*). † Normal serum.

In our study, certain modifications have made possible the consistent and reproducible passible sensitization of normal leukocytes. Serum was donated by twelve persons who were known to be allergic to Timothy pollen by the usual criteria as well as by release in vitro of histamine with their own leukocytes. The serum was stored at −70°C until used. Four nonallergic persons served as cell donors. For each experiment, nonallergic blood, collected in a siliconized syringe containing heparin, was centrifuged immediately. The buffy coat was removed, washed twice with Tyrode's solution, and separate samples were suspended in 2-ml volumes of Tyrode's solution, normal serum, or allergic serum. The suspensions were incubated in a water bath at 37°C for periods from 30 minutes to 6 hours. Timothy-pollen antigen was then added to make a concentration of 0.03 μg of protein nitrogen per milliliter; incubation was continued for one hour. After centrifugation, each cell-free supernatant solution was assayed for histamine content by a method combining two modifications of the Shore fluorometric procedure (*5*).

In each of 40 experiments, cells incubated with allergic serum released significantly more histamine after addition of antigen than those incubated with normal serum or with Tyrode's solution. These experiments included 16 different donor-donor combinations (Table 1). The effect of brief heating is shown in Table 2. All allergic sera were inactivated by heating at 56°C for 45 minutes, but their specific activity, as shown by passive transfer to skin, was not appreciably diminished. Similarly, freezing at ordinary temperatures (−10° to −18°C) did not preserve activity; consistent results were obtained only with fresh sera or with sera stored at −70°C. Apparently, complement was not necessary for sensitization, since the chelating agent, EDTA (.01*M*), did not block sensitization by active serum and fresh normal

Table 2. Histamine released from normal cell suspensions after 4 hours' incubation with allergic serum (pt. *Bo.*) and 1 hour's incubation with Timothy antigen (0.3 μg protein nitrogen per milliliter). Each sample was suspended in 3 ml of serum and 2 ml of Tyrode's solution.

Cell samples	Incubation		Histamine (μg/liter)
	4 hours	1 hour	
1–2	Serum	Antigen	17.8
3–4	Serum	No antigen	2.3
5–6	Serum*	Antigen	2.1
7–8	Serum†	Antigen	20.6
9–10	Serum*†	Antigen	2.1

* Serum was heated at 56°C for 30 min. † Cells suspended in 2 ml of allergic serum, 1 ml of fresh unheated normal serum, and 2 ml of Tyrode's solution.

serum did not restore the activity of heated allergic serum (Table 2).

Generally, we removed the allergic serum from the cells before adding antigen; this was done to reduce fluorescence contributed by serum factors (including histamine). However, histamine release after the addition of antigen was similar when the serum was left in the system.

In the first series of studies made possible by establishing the conditions essential for passive transfer in vitro, we measured the rate of sensitization of normal cells by various dilutions of allergic serum. In Fig. 1 are the results of experiments with three

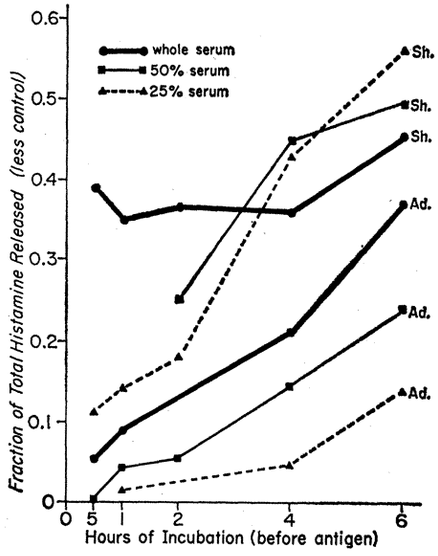

Fig. 1. Histamine released from *Va.* cells by Timothy antigen (.03 μg protein nitrogen/ml) after incubation with sera from donors *Sh.* and *Ad.* Reaction volume was 2 ml. Serum was removed and replaced with Tyrode's solution before antigen was added. Since the actual concentration of histamine released by antigen was directly proportional to the total available cellular histamine for any experimental condition, the results are expressed as the fraction of total histamine released.

dilutions of two allergic sera. The cells were from the same donor, and both the reaction volumes and cell concentration were kept constant. In each experiment, the serum was removed at the end of the first incubation and the cells were resuspended in Tyrode's solution before antigen was added. The controls, for each point, consisted of the identical steps, but with no antigen in the last hour of incubation. It is apparent that these sera differ, both in the rate of sensitization, and in the maximum amount of histamine that can be released. When serum *Sh* was diluted, the rate of sensitization was slower, yet the degree of sensitization after 4 and 6 hours incubation was greater than with whole serum. The enhancing effect of dilution has occurred consistently in repeated studies with three sera (including *Sh*); the differences are not great enough, however, to suggest the presence of a serum inhibitor. By contrast, two other sera (including *Ad*, Fig. 1) sensitized the cells at a slower rate, and histamine release was roughly proportional to the assumed antibody concentration. Fortunately, the spontaneous release of histamine during serum incubation up to 6 hours has always been relatively small so that the available histamine for antigenic release is not appreciably reduced.

The major difference between these studies and earlier ones which produced unsatisfactory results, both in our laboratory and elsewhere, was the recognition that the allergic sera were unexpectedly labile during handling and storage. Although the skin-sensitizing antibody is known to be more labile than other antibodies, its activity (as measured by passive transfer to skin) is abolished only by prolonged heating (up to 4 hours), and storage, even at room temperature, has no measurable effect for as long as 6 months (*6*).

Histamine release caused by specific antigen is an indicator of specific reactivity which has both qualitative and quantitative implications for studying human allergy. If attention is given to maintaining the activity of allergic sera, passive transfer of reactivity to other human tissues such as lung should be feasible, and precision should improve as purified antigens become available (*7; 8*).

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References and Notes

1. G. Katz and S. Cohen, *J. Am. Med. Assoc.* **117**, 1782 (1941); J. W. Noah and A. Brand, *J. Allergy* **25**, 210 (1954); P. P. VanArsdel, Jr., *et al., ibid.* **29**, 429 (1958).
2. P. P. VanArsdel, Jr., *J. Clin. Invest.* **41**, 1407 (1962).
3. V. V. Chambers, B. H. Hudson, J. Glaser, *J. Allergy* **29**, 93 (1958).
4. E. Middleton, Jr., *Proc. Soc. Exptl. Biol. Med.* **104**, 245 (1960).
5. P. A. Shore, A. Burkhalter, V. H. Cohn, Jr., *J. Pharmacol. Exptl. Therap.* **127**, 182 (1959); J. W. Noah and A. Brand, *J. Allergy* **31**, 3 (1961); L. T. Kremzner and I. B. Wilson, *Biochim. Biophys. Acta* **50**, 364 (1961).
6. J. G. Harter, *Federation Proc.* **20**, 259 (1961).
7. L. M. Lichtenstein and A. G. Osler, *ibid.* **22**, 560 (1963).
8. Supported by grant AI 01510-07 from the National Institutes of Health. We thank Mrs. Deidre Tucker for valuable technical assistance.
* Student summer research fellow, Allergy Foundation of America, 1961 and 1962.

14 June 1963

Carbohydrate Digestion in the Cockroach

Abstract. *The digestive tract of the cockroach,* Blaberus craniifer *Burmeister, contains amylase, α- and β-glucosidases, β-fructofuranosidase, α- and β-galactosidases, but no cellulase. The most active site of hydrolytic action is the midgut, followed by the foregut, hindgut, salivary gland tissue, and Malpighian tubules.*

The digestive enzymes of cockroaches have received the attention of many investigators (*1, 2, 3*). It is desirable to broaden the base for generalization regarding the distribution of carbohydrases in this group of insects and to have specific information about additional cockroach species. Accordingly, salivary glands, digestive tracts and Malpighian tubules of adult specimens of *Blaberus craniifer* Burmeister have been examined qualitatively for evidence of carbohydrase activity.

Cockroaches were reared in aquaria at about 26°C. Pablum (*4*) was provided twice a week, this being fortified periodically with Brewer's yeast. Prior to experimentation, the cockroaches were isolated and food was withheld for a minimum of 1 week, to permit their digestive tracts to be cleared of nutriment. Water was supplied continuously, but 24 hours before testing tap water was replaced by distilled water. During the final 12 hours, complete starvation was imposed.

The cockroaches were anesthetized with ethyl ether and their alimentary tracts removed. These were freed of adhering tissues and sectioned into fore-, mid-, and hindgut, and Malpighian tubules. Salivary glands were also re-