4. H. D. Patton and V. E. Amassian, *J. Neurophysiol.* 17, 345 (1954).
5. V. E. Amassian *et al.*, *Electroencephalog. and Clin. Neurophysiol.* 7, 480 (1955).
6. C. N. Woolsey and H.-T. Chang, *Research Publs., Assoc. Research Nervous Mental Disease* 27, 146 (1948).
7. H.-T. Chang, *Cold Spring Harbor Symposia Quant. Biol.* 17, 189 (1952); W. M. Landau, *Electroencephalog. and Clin. Neurophysiol.* 8, 445 (1956); R. Porter, *J. Neurophysiol* 18, 138 (1955).
8. H.-T. Chang, *J. Neurophysiol* 18, 332 (1955).
9. D. P. Purpura and H. Grundfest, *ibid.* 19, 573 (1956).
10. H.-T. Chang, *ibid.* 18, 452 (1955).

15 April 1959

Effect of Diisopropylfluorophosphate on Sulfhydryl Proteases

Abstract. Diisopropylfluorophosphate inhibited all the sulfhydryl proteases studied in our tests. This inhibition was most pronounced at *p*H 6.0. By first blocking the sulfhydryl group with *p*-chloromercuribenzoate, inhibition could be prevented. Neither cysteine nor choline gave appreciable reactivation of diisopropylfluorophosphate-inhibited bromelain.

Although the organic phosphate nerve gases are well established as specific inhibitors of certain esterases, trypsin and chymotrypsin, activated plasmin and phosphoglucomutase (*1*), no reports of specific inhibition of the sulfhydryl enzymes have been made (*2*).

In a comparative study of plant proteases we found that moderate concentrations of diisopropylfluorophosphate inhibited many preparations of bromelain (*3*), papain, and ficin. With commercial bromelain, $4 \times 10^{-4}M$ diisopropylfluorophosphate inhibited 50 percent of the protease activity within 2 hours at room temperature. Some of our fractionated bromelain preparations, on the other hand, showed only slight inhibition at high concentrations of reagent.

The effectiveness of diisopropylfluorophosphate as an inhibitor depended not only on the type of enzyme and the degree of purity of the enzyme but also on the *p*H of the solution (Fig. 1). Bromelain and papain, which presumably have similar active sites, showed an entirely different behavior at all *p*H values below *p*H 5.0. On the other hand, Rhozyme P-11, a fungal protease which does not require a free sulfhydryl group for enzymatic activity, showed a *p*H-inhibition curve which was remarkably similar to that of bromelain. These similarities and differences may provide clues to the nature of the active sites on these enzymes, or to the effect of unknown materials in these preparations which mediates the action of diisopropylfluorophosphate on sulfhydryl groups.

That the sulfhydryl group is the site actually being affected is shown by an experiment summarized in Table 1. Blocking the sulfhydryl group with *p*-chloromercuribenzoate before exposing the enzyme to diisopropylfluorophosphate gave complete protection against inhibition. Another sulfhydryl blocking technique, and one which may frequently occur with enzyme preparations, is mild oxidation. This also protected against diisopropylfluorophosphate.

Attempts to reactivate diisopropylfluorophosphate-inhibited bromelain with cysteine, choline, or a combination of the two reagents at either *p*H 5.0 or *p*H 7.0 have been only slightly successful. Cysteine regenerated no more than 10 percent of the original activity.

Our finding that diisopropylfluorophosphate, under the proper conditions, will react with sulfhydryl enzymes now makes this chemical a fairly general protein reagent. It will react directly with the nitrogen of imidazole or the hydroxyl of tyrosine (*4*); it will react directly or indirectly with amino (*5*) and sulfhydryl groups; it will react indirectly with the hydroxyl group of serine (*1, 6*).

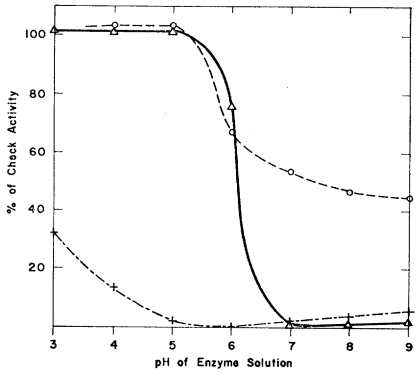

Fig. 1. Effect of *p*H on the inhibition of bromelain (solid line), papain (dashed line with crosses), and Rhozyme P-11 (dashed line with circles) by diisopropylfluorophosphate. The enzymes were incubated for 1 hour at 25°C in $1 \times 10^{-3}M$, $1 \times 10^{-3}M$, and $1 \times 10^{-4}M$ diisopropylfluorophosphate, respectively.

R. M. Heinicke
R. Mori

Hawaiian Pineapple Company, Honolulu, Hawaii

References and Notes

1. A. K. Balls and E. F. Jansen, *Advances in Enzymol.* 13, 321 (1952); F. W. Putnam, in *The Proteins*, H. Neurath and K. Baily, Eds. (Academic Press, New York, 1954), vol. IB, p. 1066; N. M. Green and H. Neurath, *ibid.* vol. IIB, 1154; L. A. Mounter and B. A. Shipley, *J. Biol. Chem.* 231, 855 (1958).
2. A. K. Balls and E. F. Jansen, *Advances in Enzymol.* 13, 321 (1952); J. A. Gladner and K. Laki, *Arch. Biochem. Biophys.* 62, 501 (1956).
3. R. M. Heinicke and W. A. Gortner, *J. Econ. Botany* 11, 225 (1957).
4. R. F. Ashbolt and H. N. Rydon, *J. Am. Chem. Soc.* 74, 1865 (1952); B. J. Jandorf, T. Wagner-Jauregg, J. J. O'Neill, M. A. Stolberg, *ibid.* 73, 5202 (1951).
5. T. Viswanatha, *Compt. rend. trav. lab. Carlsberg Sér. chim.* 30, 183 (1957).
6. We wish to thank Mrs. A. Chun, Mr. S. Nakata, and Mr. R. Sugai for technical help in running certain of these assays.

3 November 1958

Table 1. Effect of protecting the sulfhydryl group of a purified bromelain sample on inactivation by diisopropylfluorophosphate (DFP).

Treatment	Milk-clotting unit*/g	Percentage of 4660
Enzyme in *p*H 7.0 buffer (no PCMB)†	4660	100
Enzyme in 25 percent isopropylalcohol (IPA)	4520	97
Assayed without cysteine after dialysis	2620	56
Assayed with 0.005*M* cysteine after dialysis	4500	97
Enzyme in 25 percent IPA with $10^{-3}M$ DFP	706	15
Assayed without cysteine after dialysis	52	1
Assayed with 0.005*M* cysteine after dialysis	450	10
Enzyme in *p*H 7.0 buffer with PCMB†	80	2
Enzyme in 25 percent isopropylalcohol	0	0
Assayed without cysteine after dialysis	552	12
Assayed with 0.005*M* cysteine after dialysis	4900	105
Enzyme in 25 percent IPA with $10^{-3}M$ DFP	0	0
Assayed without cysteine after dialysis	486	10
Assayed with 0.005*M* cysteine after dialysis	4900	105

* Milk-clotting unit: 1 min to clot 5 ml of a 5-percent skim milk solution adjusted to *p*H 5.3 and incubated at 37.5°C. † *p*-Chloromercuribenzoate.

Influence of Adrenalectomy and Hypophysectomy on Cerebral Serotonin

Abstract. Changes in serous and encephalic serotonin in hypophysectomized or adrenalectomized rats have been observed. Adrenalectomy produces a decrease of serous serotonin and an increase of the serotonin of hemispheres, base, and medulla oblongata; with hypophysectomy, serotonin is also reduced in serum and increased only in the base and medulla oblongata.

It is known that the cerebral serotonin content can be varied by artificial means, either by stimulating the formation of

serotonin through administration of precursor drugs such as 5-hydroxytryptophan (*1*), or by preventing its degredation, by means of iproniazid, which is an excellent inhibitor of monoaminooxidase (*2*). It is also known that the administration of suitable doses of reserpine causes a reduction in the encephalic serotonin and a considerable increase in the urinary excretion of 5-hydroxyindoleacetic acid, the most important metabolite of serotonin (*3*). Chlorpromazine, meprobamate, and benactizine, on the other hand, have only slight or no effect on urinary elimination of 5-hydroxyindoleacetic acid (*4*).

I have also considered another powerful tranquilizer, hydroxyzine hydrochloride (Atarax), about which Zubiani and I have published a complete clinical study (*5*). At the present time I am studying its influence on serotonin metabolism in man (blood and cerebrospinal fluid) and in animals (blood and brain).

This preliminary note reports the modifications in encephalic and serous serotonin that have been observed in whole rats after adrenalectomy and hypophysectomy.

Sixty white rats, each weighing 200 to 250 g, were divided into three groups of 20 each. The first group was left untreated and served as controls; the second group underwent bilateral adrenalectomy; and the third, hypophysectomy. This was carried out by the transpharyngeal route. The complete removal of the hypophysis was later verified by autopsy. The animals were killed by decapitation 24 hours after the operation. The brain, which was immediately removed, was divided into four portions consisting of the hemispheres, the base, the medulla oblongata, and the cerebellum. The brain portions were then subjected to the following treatment: extraction with 80-percent acetone and filtration after 24 hours; replacement of the acetone by a fresh batch of 80-percent (5 volumes); further filtration after a second 24 hours; combination of the two filtrates and evaporation of the acetone; making up to volume with Tyrode's solution containing atropine. The serum was subjected to: extraction with 2 volumes of 100-percent acetone; filtration after 24 hours; and making up to volume with Tyrode's solution containing atropine.

Table 1. Serotonin values, in micrograms per 100 g of tissue, found in various locations in control, adrenalectomized, and hypophysectomized rats. The figures in parentheses are the percentage difference from the values found for the control rats.

No.	Rats	Location				
		Serum	Hemispheres	Base	Medulla oblongata	Cerebellum
20	Controls	50	85	97	37	0
20	Adrenalectomized	12 (−76)	195 (+129)	115 (+18)	140 (+278)	0
20	Hypophysectomized	20 (−60)	87 (+2)	154 (+58)	81 (+118)	0

For quantitative assay of the serotonin, I used rat uterus in estrus, according to Erspamer's technique (*6*). The values of encephalic and serous serotonin are expressed in micrograms per 100 g of fresh tissue.

In the control rats, the encephalic serotonin was found to be present in the quantity of 85 μg for the hemispheres, 97 μg for the base, and 37 μg for the medulla oblongata. Fifty micrograms were found in the blood serum. No trace of serotonin was found in the cerebellum, and this result agrees with the published literature. Adrenalectomized rats gave the following values for serotonin 24 hours after operation: 195 μg for the hemispheres, 115 μg for the base, 140 μg for the medulla oblongata, and 12 μg for the serum. In the hypophysectomized rats, 24 hours after operation, assay of the serotonin revealed values of 87 μg for the hemispheres, 154 μg for the base, 81 μg for the medulla oblongata, and 20 μg for the serum. These results are summarized in Table 1.

It is thus seen that adrenalectomy led to marked changes in the cerebral serotonin, which was constantly and significantly increased in the hemispheres, base and medulla oblongata, with increases of 129, 18, and 278 percent respectively, in comparison with the controls. Hypophysectomy caused a large increase in serotonin only in the base (58 percent) and the medulla oblongata (118 percent). The concentration of serotonin in the hemispheres remained practically unchanged. In both groups of animals that underwent surgical treatment, the highest increase was observed in the medulla oblongata. Both hypophysectomy and adrenalectomy produced a large reduction in the serous serotonin, which fell by 60 percent in hypophysectomized rats and 76 percent in adrenalectomized animals.

I therefore feel that it is too early to attempt to give an explanation of these results. They do, however, point to the importance of a study of the relationships between serotonin and endocrine glands (particularly the adrenals) on one hand, and mental diseases (particularly schizophrenia) on the other.

DOMENICO DE MAIO
Istituti Psichiatrici Provinciali,
Milan, Italy

References

1. S. Udenfriend, H. Weissbach, D. F. Bogdanski, *Ann. N.Y. Acad. Sci.* 66, 602 (1957); *J. Biol. Chem.* 224, 803 (1957); S. Udenfriend and H. Weissbach, *Proc. Soc. Exptl. Biol. Med.* 97, 748 (1958).
2. B. B. Brodie, A. Pletscher, P. A. Shore, *Science* 122, 68 (1955); *J. Pharmacol. Exptl. Therap.* 116, 9 (1956); M. Chessin, E. R. Kramer, C. C. Scott, *ibid.* 119, 453 (1957).
3. P. A. Shore, S. L. Silver, B. B. Brodie, *Science* 122, 284 (1955); S. Udenfriend, E. O. Titus, *Federation Proc.* 13, 411 (1954).
4. F. M. Berger, G. L. Campbell, C. D. Hendley, B. J. Ludwig, T. E. Lynes, *Ann. N.Y. Acad. Sci.* 66, 686 (1957).
5. A. Zubiani and D. De Maio, Comun. VI Congr. Nazl. Soc. Ital. Gerontol., Napoli, 1956. "Senigaglia V," in *Minerva Med.* 2, 2985 (1957); *Psychotropic Drugs* (Elsevier, Amsterdam, 1957), pp. 170, 547.
6. V. Erspamer, "Enteramina," *Rend. Sci. Farmitalia* 1954, 122 (1954).

6 August 1958