forms having relatively weak fundamentals.

The information now available provides a fairly precise description of the time sequence of events in the retina during flicker fusion. Under the specific conditions mentioned above, flicker fusion thresholds do depend on waveform, contrary to previous opinion (*1*), and not upon one component alone (*3*, *5*).

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5. A paper combining these considerations with results of H. deLange (*1*) and C. Enroth [*Acta Physiol. Scand.* **27**, suppl., 100 (1952)] is in preparation. Thanks are due to J. Kohut for assembling the apparatus and taking the data. The efforts of his patient subjects are also gratefully acknowledged.

1 February 1960

Is Reserpine Tranquilization Linked to Change in Brain Serotonin or Brain Norepinephrine?

Abstract. Reserpine, when administered to animals stressed by exposure to cold, does not induce sedation or appreciably lower brain serotonin, but markedly lowers brain norepinephrine. Reserpine in cold-exposed hypophysectomized rats elicits sedation and releases both amines equally. The results support the view that the tranquilizing action of reserpine is not related to brain norepinephrine loss but rather to change in the level of brain serotonin.

The tranquilizing action of reserpine, originally linked to the release of brain serotonin, is now often ascribed to the loss of brain norepinephrine. From studies based on bioassay, Kärki and Paasonen (*1*) concluded that Raunescine, in doses which release norepinephrine but not serotonin in the brain, has a sedative effect in rats. But, using fluorimetric methods, we found that various sedative doses of Raunescine lower serotonin and norepinehrine levels to the same extent (*2*). Pletscher *et al.* (*3*), on the basis of studies in mice with two benzoquinolizine derivatives, proposed that the sedative action of reserpine is associated with loss of brain norepinephrine. They reported that in 1 hour the potent tranquilizer, Ro 4-1284, releases more brain norepinephrine than does the weak tranquilizer Ro 4-1398, while the two drugs release serotonin to the same extent. However, we found that whereas in 20 minutes Ro 4-1284 releases much more serotonin than does Ro 4-1398, in 1 hour the difference in serotonin levels disappears because the action of the former compound is brief and brain serotonin forms rapidly (*2*). Thus, the results with Raunescine and the benzoquinolizines do not make it possible to associate sedation with either one of the amines.

Contrary to findings of many other workers are those of Sheppard and Zimmerman (*4*), who reported that the subcutaneous injection of small doses of reserpine (0.1 mg/kg) into female guinea pigs causes in 20 minutes a rise of 75 percent in brain norepinephrine level, and in 2 hours a rise of 45 percent in heart norepinephrine level. After 2 hours they found a small decline in brain serotonin level. These authors measured norepinephrine fluorimetrically by a procedure in which filters are used for isolating the activation and fluorescent light bands. Because of the relatively wide spectral bands of filters, the validity of the values thus obtained is contingent on proof that the method for norepinephrine determination is specific.

This is especially relevant in view of the presence of reserpine and its metabolites in the body. However, no evidence is offered for the specificity of the method. The experiments were repeated in this laboratory (*5*) with a fluorescence spectrometer, which permits the use of narrow spectral bands. With methods of proved specificity we have shown that the administration of 0.1 mg of reserpine per kilogram to female guinea pigs at no time causes a rise in brain or heart norepinephrine levels. Brain levels of norepinephrine and serotonin decline at the same rate and to the same extent.

A study of the phenomenon, noted by Garattini and Valzelli (*6*), that administration of reserpine to cold-exposed rats causes no sedation and no decline in the level of brain serotonin, led us to discover that, in animals subjected to stress, administration of reserpine considerably depletes the amounts of norepinephrine in the brain but does not elicit sedation or appreciably change the content of brain serotonin.

Rats (males, Sprague-Dawley, weighing 150 to 180 gm) after 4 hours' exposure to cold (4°C), were injected intraperitoneally with 1 mg of reserpine per kilogram. The animals were then kept in the cold for an additional 4 hours, during which time they gave no evidence of sedation. The rats were then decapitated, and the brains were analyzed for norepinephrine and serotonin by fluorimetric methods (*7*). As shown in Table 1, the reserpine released considerable amounts of brain norepinephrine but affected brain serotonin levels only slightly. Exposure of rats to cold for 8 hours without reserpine administration resulted in no change in the amine levels. Reserpine given to rats at room temperature (22°C), or to rats exposed only briefly to cold, elicited marked sedation and released both amines. Experiments with rabbits gave similar results.

A close association between the appearance of sedation and the release of brain serotonin was shown by experiments in which rats were exposed to cold for 4 hours, given reserpine, and then brought to room temperature. The levels of brain serotonin then slowly declined, and evidence of sedation appeared only when the serotonin level had declined by about 50 percent.

The possibility that "stress," produced by exposure to cold, prevented the decline in brain serotonin and the sedative action of reserpine was tested by administering the drug to cold-exposed, hypophysectomized rats. Under these circumstances, reserpine elicited sedation and released both amines. Hypophysectomized rats exposed to cold for 8 hours without reserpine showed no change in brain amine levels.

These studies suggest, but do not prove, a causal relation between the release of serotonin and the tranquilizing actions of reserpine. Other lines of evidence also indicate that the sedative action of reserpine is associated with changes in brain serotonin rather than changes in brain norepinephrine. For example, studies from our laboratory show that small doses of Su 5171 (dimethylaminobenzoyl methylreserpate) release relatively little brain serotonin in rabbits; the animals give no evidence of sedation, despite a marked decline in brain norepinephrine (*2*). Finally, recent reports indicate that the norepinephrine loss induced by reserpine does not lower sympathetic discharge from the central sympathetic system (*8*) and may even increase the outflow (*9*).

Table 1. Brain levels of serotonin and norepinephrine (± standard error) in rats exposed to cold stress. The animals were given 1 mg of reserpine per kilogram, intraperitoneally. Figures in parentheses refer to number of experiments. The brains of three animals were pooled in each experiment.

Treatment	Serotonin content (μg/g)	Norepinephrine content (μg/g)	Sedation
None	0.45 ± 0.02 (15)	0.49 ± 0.02 (15)	
Reserpine at 22°C	0.16 ± 0.02 (15)	0.16 ± 0.02 (15)	Yes
Brief (2 min) cold-exposure followed by reserpine at 4°C	0.19 ± 0.02 (4)	0.19 ± 0.03 (9)	Yes
Long (4 hr) cold-exposure followed by reserpine at 4°C	0.36 ± 0.02 (9)	0.23 ± 0.03 (9)	No

These results are in agreement with the possibilities that, on the release of stored amines in brain, *total* amine levels, but not necessarily *free* amine levels, are lowered, and that the central effects of reserpine depend on the levels of free amine at the receptor sites (*9*). Because of the very rapid synthesis of serotonin (*10*) as compared to norepinephrine (*11*) in brain, the effects of free serotonin may predominate after reserpine action.

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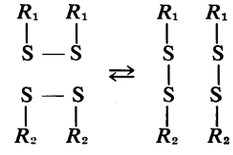
18 December 1959

Disulfide Interchange by Ionizing Radiation

Abstract. The irradiation of a solution of two symmetric disulfides produces detectable amounts of the nonsymmetric mixed disulfide. The effect is abolished by the addition of ethylmaleimide. The finding indicates that radiation causes a disulfide opening and recombining process which may be of radiobiological interest.

The sulfur groups present in the structures of the cell have generally been considered some of the loci most vulnerable to radiation. The nature of the changes induced by radiation in these groups is not yet completely understood. In considerating the possible extension of studies of simple systems to more complex ones, chemical changes in sulfur compounds of low molecular weight resulting from radiation have been frequently investigated. Researches have been focused on the degradation of these compounds (*1*) or on the oxidation (*2*) and the reduction (*2, 3*) of their sulfur portion. The occurrence of a less destructive action of ionizing radiation on sulfur compounds—that is, disulfide interchange—is reported here; though less destructive, this phenomenon might equally effect a disorganization of disulfide-containing substances.

Disulfide interchange was studied in a system composed of cystamine and N-diformylcystine. This system permitted the use of compounds soluble over a large *p*H range and of paper electrophoresis for the detection of the interchange product. As a matter of fact, cystamine and diformylcystine, having different dissociation, migrate in opposite directions, while the mixed disulfide produced by the interchange



($R_1 = -CH_2-CH_2-NH_2$;
$R_2 = -CH_2-CH(NHOCH)-COOH$)

having an intermediate charge, migrates at an intermediate rate.

Four hundred micromoles of cystamine dihydrochloride (*4*) were dissolved in 4 ml of the same acetate buffer that was used for electrophoresis; 400 μmole of N-diformylcystine was suspended in 4 ml of water and brought into solution by the addition of solid sodium carbonate. The two solutions were mixed (final *p*H, 3.8 to 4.0) and irradiated for a suitable length of time with a Philips 50-kv x-ray source at a distance of 1 cm. The magnitude of the radiation dose was checked by a ferrous sulfate dosimeter. At any desired time a 0.02-ml sample of the solution was spotted at the center of a strip of Whatman 3-mm filter paper. The paper was then wetted with a 0.45*M* sodium acetate buffer of *p*H 3.75 (ionic strength, 0.05). Electrophoresis was performed in the same buffer, with a potential of 300 volts (about 10 volt/cm of paper), for 30 minutes. After the paper was dried the compounds were located by spraying with the Folin-Marenzi reagent, with bisulfite added according to a procedure previously devised for the detection of disulfides (*5*).

The results are shown in Fig. 1; they indicate that under the action of radiation a new disulfide compound, which in electrophoresis does not migrate, is promptly produced in amounts related to the radiation dose. In order to identify the new compound, larger amounts of it were prepared by large-scale electrophoresis. As was to have been expected from the mixed disulfide formed by interchange between cystamine and diformylcystine, upon oxidation with H_2O_2 and ammonium molybdate (*6*), followed by hydrolysis in 1*N* HCL for 2 hours at 100°C, the new compound yielded taurine and cysteic acid, which were detected by paper chromatography.

The radiation-induced disulfide exchange was observed also at neutral *p*H and with concentrations of the reactants other than those reported above. Be-

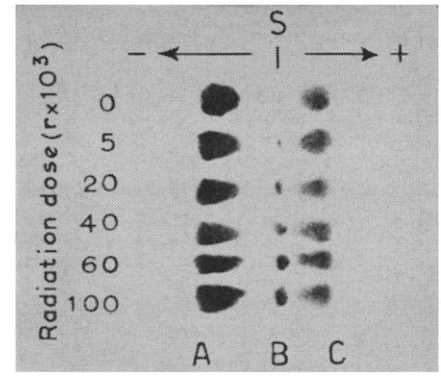


Fig. 1. Disulfide interchange by radiation. Paper electrophoresis of a solution of equimolar amounts of cystamine and diformylcystine after irradiation with increasingly large doses of x-rays. *S*, starting point; *A*, cystamine; *C*, N-diformylcystine; *B*, mixed disulfide. Spots were developed by spraying with the Folin-Marenzi reagent for disulfides.

cause of the slow spontaneous exchange (*7*), which tends to mask the results, the described conditions are the most suitable for a clear-cut demonstration of the radiation effect. The presence of an excess of N-ethylmaleimide abolishes the exchange; this indicates that the reaction probably proceeds through the temporary opening of the disulfide bonds with liberation of thiol groups. However, the nitroprusside test for thiol groups, carried out soon after irradiation of the solutions, was found invariably negative.

By slightly modifying the experimental conditions, so as to solubilize cystine (solution of the disulfides in final 0.2*N* HCl), the exchange can be observed also between cystine and cystamine.

Two conclusions should be drawn from the above results. First, the radiation-induced exchange can be expected to occur also within a single molecular species of disulfide. This effect of radiation, which is not followed by analytical change of the compound, might dissipate radiation energy, thus contributing to the reduction of the damaging action of radiation observed in the presence of disulfides. Second, present results, if extended to proteins, might provide another approach to the understanding of the disorganization of secondary and tertiary structures of disulfide-containing proteins brought about by radiation. In this connection, the disulfide interchange which has been reported to occur in the course of the chemical denaturation of proteins (*8*) and polypeptides (*9*) is highly suggestive (*10*).

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