acceptor, such as methylene blue or cytochrome *c* (*4*). The possibility was explored that ferritin might be reduced as a result of xanthine dehydrogenase activity under conditions of lowered oxygen tension. When ferritin was added to an anaerobic mixture of xanthine oxidase, hypoxanthine, or xanthine, and α,α′-dipyridyl to act as a trapping agent, the ferrous iron content of the system was increased 2.6 times. In this anaerobic reaction, the first reduction of ferritin iron must occur by virtue of its action as an electron acceptor, for no uric acid is formed in the absence of ferritin.

The reduction of inorganic iron by xanthine oxidase has been reported by Weber *et al.* (*5*) to be dependent on the formation of H_2O_2. The presence of oxygen in our system stimulated reduction of ferritin iron. This may be explained by the formation, in oxygen, of increased amounts of uric acid, which reduces more ferritin. The addition of crystalline catalase to the aerobic reaction mixture caused a further stimulation of ferritin reduction, presumably by protecting the enzyme against inactivation by H_2O_2. Therefore, H_2O_2 is apparently not involved in the reduction of ferritin iron.

A homeostatic mechanism for the regulation of plasma iron levels is suggested by these results. In addition, preliminary experiments indicate the presence in aerobic liver of a system (as yet uncharacterized) that is capable of oxidizing ferrous ferritin to the ferric state. In the normal aerobic liver, ferrous ferritin, formed as a result of the activity of xanthine oxidase on relatively low levels of hypoxanthine and xanthine, would be largely reoxidized. In the hypoxic liver, as a consequence of dehydrogenase activity on increased quantities of hypoxanthine and xanthine, more ferritin is reduced to the ferrous form and cannot be reoxidized. This results in increased levels of plasma iron.

In vivo confirmation of the role of uric acid in the release of ferritin iron to the plasma has been obtained from preliminary experiments carried out with S. Baez and S. G. Srikantia in our laboratory, using rats subjected to hemorrhagic shock. In such animals, we observe an increased plasma uric acid level, which cannot be accounted for solely by inhibition of kidney function caused by lowered blood pressure. Similar increases in plasma uric acid have been reported by others (*6*) without explanation of the mechanism involved.

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28 September 1956

Criteria for Assessing Effects of Drugs on Posttetanic Potentiation

There is considerable evidence that posttetanic potentiation (PTP) is a general synaptic property and is a normal consequence of synaptic excitation (*1–5*). Modification of the potentiating process by drugs (for example, *6–8*) provides clues to the nature of the phenomenon as well as information concerning the mechanisms of action of the drugs. Because of anatomical and physiological limitations on size of the efferent neuronal pool (in monosynaptic pathways of the spinal cord and in sympathetic ganglia), it is possible in some circumstances to modify profoundly the potentiating process without affecting the degree of PTP of synaptic transmission as tested in the usual manner, namely, by comparison of maximal synaptic discharges before and after tetanic stimulation. Jefferson and Benson (*9*) have shown that under certain conditions the total relevant motoneuron pool may be discharged during maximum PTP of the monosynaptic (2N) pathway of the spinal cord. Furthermore, at high levels of excitability such potentiation may be more than sufficient to lead to excitation of available motoneurons.

Figure 1*A* shows input-output relations in the 2N pathway of the spinal cord in the resting state (*10*) and during the period of maximum PTP (*11*) at various levels of reflex excitability as controlled by spinal cord temperature (*12*). With increasing size of maximum discharges at rest (*Y* intercepts of curves *a, b, c,* and *d*) the maximum potentiated discharges increase to a ceiling (curves *c′* and *d′*) imposed by limitations on available postsynaptic neurons. Curves representing PTP with time after tetanus at the corresponding levels of excitability show that the maximum posttetanic discharges must be the same for all curves (*c′ c″*; *d′, d″*) on this ceiling.

Therefore, when the input-output

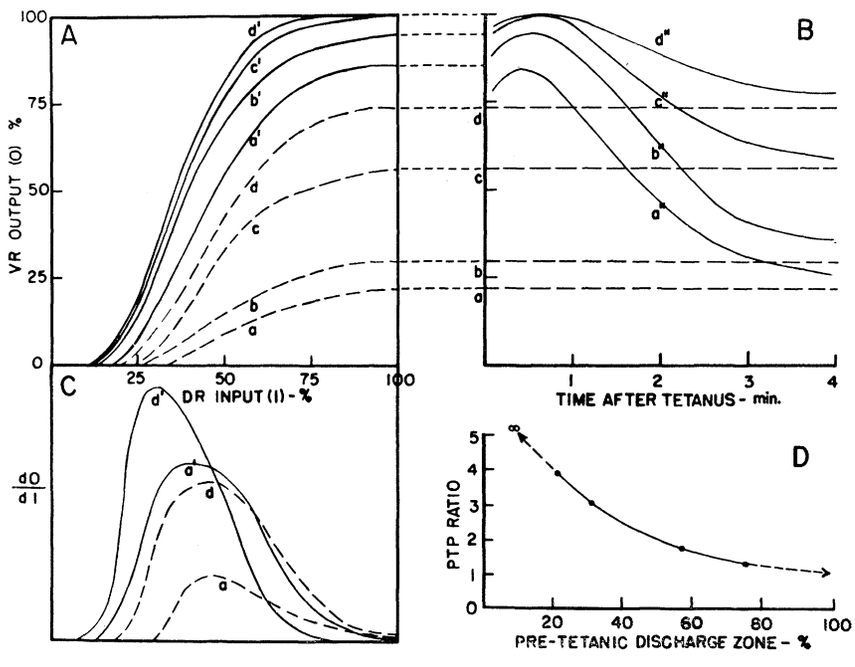

Fig. 1. Various aspects of PTP in the monosynaptic pathway of the spinal cord studied at four levels of excitability. (*A*) Experimentally determined input-output curves at rest (*a–d*) and during the peak of PTP (*a′–d′*). Excitability was altered by varying spinal cord temperature. Temperatures for curves *a–d* were 36°C, 35°C, 32°C, and 30°C, respectively. Unanesthetized spinal cat, DR-VR (S_1) preparation. (*B*) Curves illustrating time-course of PTP at the various levels of excitability. (*C*) Curves illustrating distributions of synaptic thresholds for several of the curves in *A*, obtained by graphical differentiation of corresponding input-output curves. Areas under curves are proportional to respective discharge zones in *B*. (*D*) relationship between PTP ratio and discharge zone at rest. Values plotted are those obtained from the *Y* intercepts of *A*.

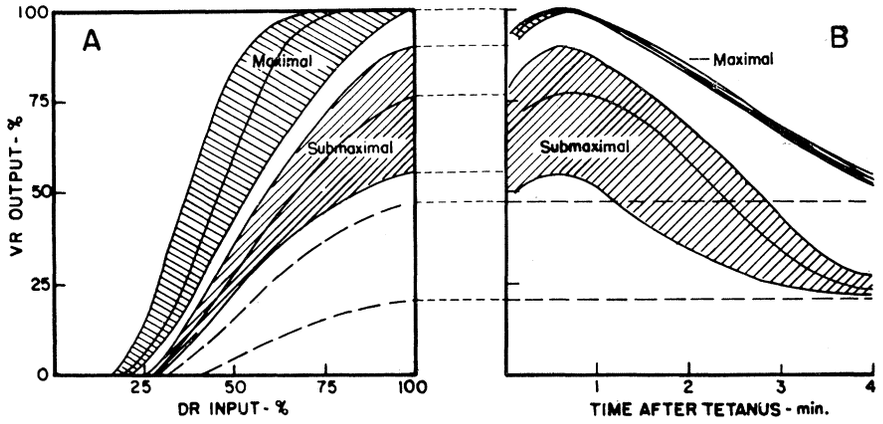

Fig. 2. A comparison of the ways in which changes in the potentiating process are expressed by input-output curves (*A*) and curves representing the time-course of PTP (*B*). Two levels of excitability are shown: "Maximal" in which the potentiated discharge reaches the maximum over all degrees of PTP and "submaximal" in which the potentiated discharge never reaches the maximum. The curves are generalized from 31 spinal cord experiments.

curves (*A*) during PTP tend toward this ceiling, increases or decreases in the potentiation process, as revealed by these curves and reflecting changes in synaptic threshold (*C*), may not be fully indicated by the method of testing employed in *B*. Curves expressing these relationships are shown in Fig. 2. In the "maximal" region, changes which fail to lower the input-output curves from the ceiling give no evidence of an alteration in PTP when tested in the usual manner, except for small changes in the time-course of potentiation.

The curves of Fig. 2 are based on results obtained in a study of the effects of diphenylhydantoin (Dilantin) on synaptic transmission (*7, 8, 13*). Dilantin has negligible effects on the level of reflex excitability of the spinal cord and on synaptic threshold at rest. On the other hand, the drug consistently produces a shift to the right in the input-output curves obtained during PTP. The expected decrease in PTP, however, is revealed only when testing is performed in the "submaximal" zone.

In examining means of avoiding the pitfall just described, consider first the simplest situation, that in which the level of excitability at rest is not influenced by the drug. It is apparent that effects of drugs on PTP can be assessed in three ways: (i) Input-output relations can be determined directly, at rest and during PTP. (ii) PTP may be studied in the usual fashion after it has been determined that testing is being carried out in the "submaximal" zone. If the initial level proves unsatisfactory (that is, in the "maximal" zone), then a more desirable level may be found by raising the temperature (see, for example, Fig. 1*A*) or, alternatively, by reducing the number of afferent fibers subjected to stimulation. (iii) PTP may be tested in the usual way but following a tetanus with either frequency or duration reduced so that the maximum potentiated discharge is less than that produced by maximal tetanic parameters (*2*).

A further difficulty in interpreting effects on PTP is introduced if the drug increases or decreases the discharge zone at rest, inasmuch as equivalent degrees of PTP are not to be expected at different levels of excitability (*12, 14*). Figure 1*D* shows the relationship between PTP ratio and maximal discharge zone at rest (pretetanic) which holds for the data of Fig. 1*A*. The additional complication of change in discharge zone can be dealt with in several ways. Qualitative statements regarding the effect of a drug are justified if both PTP ratio and discharge zone at rest are influenced in the same direction by the drug (Fig. 1*D*).

For more quantitative evaluation of the effect of the drug it is often possible to vary excitability (for example, by changing temperature) so that the discharge zone is restored to the control level. PTP may then be compared with the control determination. In altering level of excitability to restore the initial discharge zone the assumption is implicit that the procedure employed to vary excitability (for example, temperature) does not specifically affect the potentiating process. The soundness of conclusions derived in such experiments depends on the validity of this assumption.

The foregoing considerations refer specifically to 2N pathways of the spinal cord. For the most part, however, they apply also to sympathetic ganglia; the postsynaptic neuronal pool is limited, and a similar relation exists between PTP ratio and discharge zone at rest (*3, 13*). Furthermore, Dilantin reduces PTP at this site only when testing is in the "submaximal" zone. It is probable that the considerations discussed apply in a general way to PTP in polysynaptic pathways of the spinal cord (*4*) and in all other synaptic systems in which, under the experimental conditions employed, limitations on efferent pool size exist.

It is therefore apparent that drugs studied for modification of PTP must be tested in a manner capable of revealing an effect if one exists. In addition, if the agent changes the level of excitability, a nonspecific effect on PTP and on PTP ratio is to be expected. Arguments for a specific effect of a drug on the potentiating process must take account of these relationships.

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8 October 1956

Correction

Two corrections should be made in the item "Blood groups and disease" [*Science* 124, 674 (12 Oct. 1956)] which reports a paper on associations between blood groups and disease read to the British Association for the Advancement of Science.

The three diseases for which the evidence is overwhelming are (i) duodenal ulcer, (ii) gastric ulcer, (iii) cancer of the stomach. As is stated, the ulcers are commoner in persons of group O, and cancer of the stomach in persons of group A. There is fairly strong evidence in connection with diabetes mellitus and pernicious anemia; but the incidence is raised in group A, not groups O and B.

It should perhaps be added that practically all the data so far come from Western Europe and North America, and the associations might be different or absent in other peoples.

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