THRESHOLD EFFECTS OF TOXIC SUBSTANCES ON SOME FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM

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The development of production techniques and health engineering is gradually eliminating the dangers from acutely active harmful factors. The study of the reaction of the organism to the chronic action of environmental factors of less intensity is, therefore, becoming one of the central problems in hygiene. This change is also leading to more exact and better hygienic standards. Although the problem is of the utmost importance to industrial toxicology, methods for its solution have not yet received adequate attention from either the practical or theoretical viewpoint.

The methods so far used are suitable for the examination of clear-cut cases of intoxication in the clinic, or for the pharmacological evaluation of the toxic effects of rather large doses, but cannot indicate the fine changes produced by the chronic threshold effects of toxic substances. The maximum allowable concentrations are generally given empirically and have no adequate objective foundation.

As to the theory of threshold toxicity, the essential problem is concerned less with obviously pathological processes than with those which are more or less physiological. From this point of view it is necessary to apply several physiological principles, such as the different effects of a particular substance administered in small and large doses (Arndt and Schulze's rule), or the two-phase effectiveness, in the sense of the theory of parabiosis of Vvedensky, non-specific and summation effects, *etc.* The threshold action is influenced to a great extent by secondary factors, by the actual functional state of the organism, and its individual reactivity, especially by the reactivity of the sensitive neurohumoral system. With different levels of concentration or doses, the qualitative side of the effect can also change to some extent.

We can study the direct or indirect effects of materials on the physiological processes at three qualitatively different levels, *i.e.* from the point of view of their influence on

(i) elementary biochemical and biophysical phenomena;

(ii) the functions of individual organs and systems;

(iii) the adjustment of the organism as a whole to its environment.

With massive doses the changes can be more apparent at one of these levels, *i.e.* on that in which it acts in a so-called specific manner as, for example, with cytotoxic, organotoxic and neurotoxic substances; with low concentrations of many kinds of substances it appears that the changes first become apparent in the neurohumoral regulation systems, especially in the higher nervous functions, the basis of whose activity is reaction to the smallest changes in environment.

The methods developed in the study of the physiology of the central nervous systems and higher nervous activity (including the study of behaviour) have been used lately not only for ascertaining neurotoxicity, but also as one of the " integral indicators of toxicity ". A number of good results have been obtain in a relatively short period, but one can see that by no means all possibilities for the study of threshold and chronic toxicity have yet been used. In this paper we have concentrated on this methodological aspect of the subject.

In contemporary physiology there are two effective ways of studying the central nervous mechanism:

(i) the study of reflex functions (including higher nervous functions or behaviour);

(ii) the study of electrophysiological properties.

We have used both ways, which are complementary.

The influence of various toxic substances on higher nervous activity was studied in various laboratory animals by the method of conditioned reflexes, using various set-ups, and with the help of behaviour studies, or spontaneous motor activity.

We also traced the active electrophysiological phenomena, *i.e.*, the recording of E.E.G. and the passive electrophysiological characteristics, *i.e.*, we measured the electrical excitability of the cortical motor centres and also the simple reflex functions exemplified by the flexor reflex according to Zakusov and Lyublina.

To indicate the sensitivity of these procedures some examples of our results will now be given. While with a carbon disulphide concentration of 2000 μg/l. there are no visible effects in the weight curve or death rate (Marhold) of rats exposed for 6 months (6 hours a day), we can see, even after a few days' exposure, visible changes of various degrees in different parameters of conditioned behaviour up to the disappearance of positive conditioned reflexes (depending on the typological properties of the nervous system). With a concentration of 150 μg/l. there were no changes of conditioned reflexes, even after many months of exposure. After a week's exposure to carbon disulphide, the E.E.G. of a rabbit revealed threshold changes even with a concentration of 50–80 μg/l. (*i.e.* with a concentration complying with the American standard), and, after a week's exposure to a concentration of about 400 μg/l., the changes lasted for 1–2 weeks. Apart from the changes revealed in the tests mentioned, we noticed no clinical effects of exposure.

For exposure to carbon disulphide, the E.E.G. of a rabbit was a more sensitive test than food conditioned reflexes in rats. With trichlorethylene the opposite was true: the first changes in the E.E.G. appeared only after high doses under subnarcotic states in the behaviour of the rabbit, whereas with rats statistically significant changes of the conditioned reflexes were noticed after chronic exposure, even with a concentration complying with the Czechoslovak hygienic standard, *i.e.* 400 μg/l. The greatest changes were in the discriminative behaviour (differential inhibition). In addition to simple food reflexes, consisting of running to the feeding trough, the Voronin-Skinner method, in which rats learn to press a pedal to obtain food, was used.

The question of the different sensitivity of the different kinds of conditioned and unconditioned reflexes has been discussed several times, and it is known, for instance, that the defensive reflexes are less sensitive than the food reflexes. While we observed marked changes in the discriminative food reactions of rats with a trichlorethylene concentration of 400 μg/l., Grandjean and Bättig did not observe any changes in conditioned defensive reflexes with concentrations several times higher. Their results were influenced by the fact that they did not use discriminative behaviour and it is differential inhibition that is the first to alter. Dews showed with pentothal and chlorpromazin that, by reinforcing conditioned reactions in two different ways, using the same apparatus, changes can be observed which are either parallel or opposed to one another. It is, therefore, very important to choose adequate indicators, and to keep to a standard procedure, in order to gain a true picture of the methods and to be able to compare the results of various laboratories.

For various conditioned reflexes and behavioural studies, we have constructed an apparatus with programmed control and recording. This enables much experimental information to be obtained under strictly standard conditions. It eliminates the human factor, and thus avoids error due to differences in the skill of the experimenter. The results can be recorded either directly in the digital form or on perforated tape, a procedure which speeds up further statistical work.

A further way to evaluate the excitability of the central nervous system is by testing the threshold of electrical stimulation. One of the most suitable systems for this purpose is the motor zone of the cerebral cortex: electrodes for chronic experimentation may easily be implanted over it and its stimulation results in a directly visible effect. At first, we correlated the increase in the cortical motor threshold (for rhythmic stimulation by 50 c/s sine wave current) during barbiturate anaesthesia with the depth of anaesthesia (determined by the reflex activity and E.C.G.). Later, we tried to use this method for testing industrial poisons of narcotic or convulsive activity (*e.g.* trichlorethylene and parathione) in rabbits. In guinea-pigs the neurotoxic activities of benzene, toluene and xylene were compared: the narcotic activity increases markedly in this homologous series, while the convulsive one appears only in benzene.

To a smaller extent we tried to utilize examinations of some central nervous functions of men (motor conditioned reflexes, association tests). To determine diagnostically the preclinical disturbances of a man exposed to trichlorethylene, the changes in higher nervous activity were correlated with Fujiwara's reaction in urine; in a number of cases these changes appeared sooner than the positive biochemical reaction. To follow the threshold or the reflex influence of harmful substances on human subjects, we examined a number of physiological methods, *e.g.* the measurement of the electrical sensitivity of the eye.

In conclusion, we should like to state that the physiological approach opens up new, rich, perspectives for toxicological hygienic research, mainly in the field of threshold effects of harmful substances, but that it is not claimed that this approach is of universal application, or that it should replace chemical, hygienic and clinical analysis.