COMPARISON OF TWO MORPHOMETRIC METHODS FOR ASSESSING THE DEGREE OF FATTY DEGENERATION OF THE LIVER

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The use of quantitative methods of estimating the morphological substrate of various pathological processes has been due primarily to the need for maximal objectivization of data. In toxicologic practice the degree of fatty degeneration caused by hetertotropic poisons is usually estimated by subjective grading, followed by frequency processing of the data. This method is very laborious, it is not free from subjectivism, and it needs to be improved. Accordingly a new semiautomatic method of activation of the degree of fatty degeneration of the liver using the TAS instrument (Leitz-Wetzlar, West Germany) has been developed and tested.

The investigation described below was carried out with the parallel aim of determining the degree of fatty degeneration of the liver by the two methods specified above at different times after administration of a single dose of CCl_4 in different concentrations.

EXPERIMENTAL METHOD

Male Wistar albino rats weighing initially 200-240 g were used, eight to ten animals in each experimental group. The animals were exposed for 4 h, once only, to CCl_4 in concentrations of $25,700 \pm 250$, 2010 ± 60, and 30 ± 7 mg/m^3. The animals were killed on the day of inhalation, and also, 2, 4, 8, and 14 days after exposure. Material for investigation was taken with observance of the demands for systemic morphometric analysis. Pieces of liver selected for investigation by the "fields" method [1] were fixed in 10% neutral formalin solution. Sections 40-50 μ thick were cut on a freezing microtome. Sections for further telemetric investigation were chosen from serial sections with the aid of a random numbers table. Sections were stained with Sudan black and analyzed with the aid of a television system, in accordance with a program for estimating the bulk fraction of sudanophilic granules in the liver tissue.

The results of preliminary analysis showed that it is sufficient to analyze 50 fields of vision, for the coefficient of variation was under 5%. The necessary number of fields of vision was determined by the equation $n = \dfrac{400\,D}{M^2}$, where M is the arithmetic mean and D its dispersion. In the present case $n = 47$. Choice of field of vision was made manually by a random numbers table. Telemetric analysis was carried out with a TAS instrument (Leitz-Wetzlar, West Germany) in which the version based on the principle of point counting with points of zero thickness was used. Information on the serial numbers of the preparation, group, and series of experiments was introduced manually into the memory of an Olivetti minicomputer. The total number of measurements made and the arithemetic mean value of the specific volume of the sudanophilic fraction in a section studied were estimated automatically and also stored in the computer. After analysis of the whole mass of the preparation, automatic statistical analysis was carried out, yielding information on the arithmetic mean value, its dispersion, standard deviation, error of the arithmetic mean, and coefficient of variation was obtained. A comparative statistical analysis was carried out, using the Student—Fisher test, between experimental groups in each series of the investigation. Visual gradation assessment was carried out on the same preparations, with strict observance of all demands of systematic histometric analysis. The results obtained by the two methods were compared with those obtained by the use of frequency characteristics.
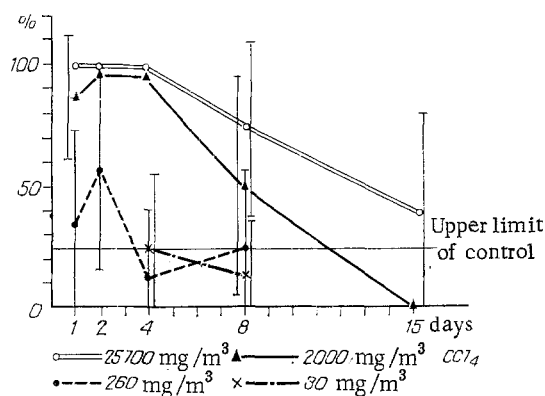
Fig. 1. Relative number of animals
with increased fat content in liver
(<0.1%; semiautomatic method). Ab-
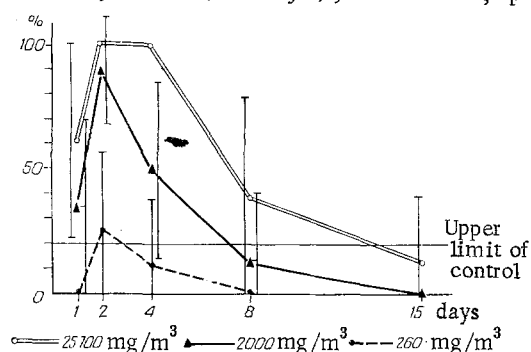scissa, time (in days); ordinate, percentage.

Fig. 2. Relative number of animals
with fat content in liver assessed
on "average-much" scale (subjective
gradation). Abscissa, time (in days);
ordinate, percentage.

EXPERIMENTAL RESULTS

The results of frequency analysis of data obtained by subjective gradation assessment
of the degree of fatty degeneration of the liver are given in Fig. 1. No sections of the
liver with a high fat content ("much" on the conventional scale) were found in the combined
control group (n = 68). No fat or only "a little" was present in the liver of 95% (63 of 68)
of the animals. The number of animals with an "average" fat content varied in individual
control groups from 2 to 20%. The highest relative number of animals with signs of fatty
degeneration of the liver (20%) observed in the control was taken as the upper limit of normal.

The most marked and persistent fatty degeneration of the liver was observed after a
single exposure to CCl_4 in a concentration of 25,700 mg/m^3, with maximal damage on the 2nd-
4th day and normalization by the 14th day. Exposure to CCl_4 in a concentration of 2000 mg/m^3
led to fatty degeneration in fewer animals, less severe at all times of investigation, and
returning to normal sooner (by the 8th day). In a concentration of 260 mg/m^3 CCl_4 gave rise
to a near-threshold effect: a very small increase in the relative number of animals with
fatty degeneration on the 2nd day after a single exposure and permanent normalization there-
after. In a concentration of 30 mg/m^3 CCl_4 caused no increase in the number of cases of fatty
degeneration in the experimental animals.

Results of frequency analysis of data obtained by semiautomatic assessment of the degree
of fatty degeneration of the liver in the same animals are given in Fig. 2. Analysis of the
combined control group (n = 59) showed that the bulk fraction of sudanophilic granules varied
from 0 to 4.6% and that approximately 80% of sections analyzed (48 of 59) contained a fraction
of sudanophilic granules in a volume of under 0.1%. The relative number of cases exceeding
this limit in individual control groups varied from 5 to 25%. The largest relative number of
animals with signs of fatty degeneration observed in the control (25%) was taken as the upper
limit of normal.

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On semiautomatic assessment of the degree of fatty degeneration caused by a single inhalation of CCl_4, basically the same patterns were found as by subjective gradation assessment described above: the maximal effect following exposure to CCl_4 in the highest of the concentrations tested, a threshold response to concentration of 260 mg/m^3, and no effect when CCl_4 was inhaled in a concentration of 30 mg/m^3.

Comparison of the results of evaluation of the degree of fatty degeneration by two morphometric methods thus showed that the results obtained evidently coincided, showing that the methods are adequate for the purpose. The method of semiautomatic evaluation of the degree of fatty degeneration of the liver which the authors have developed and tested not only yields more objective data, but it saves considerable time. The throughput of the instruments is such that 200 sections can be analyzed by it in the course of one working day, whereas the largest number of sections which can be analyzed by visual methods is 20 to 30 per day.

The next task is to reduce the margin of error (which at present may amount to 15-20%) of the method and to reduce the variability of the results (C_4 in the control was 30%). A solution to the problem is being sought by the development of selective contrast stains for the substrate and standardization of the conditions of preparation of the histological sections.

When comparatively thick sections are stained with Sudan black it is impossible to avoid pale gray background staining, the intensity of which is largely determined by variability in the case of a small or average fat content in the liver. Irregular thickness of the sections due to imperfect microtomy technique (in our experiments variation in the thickness of the sections ranged from 7 to 40 μ in the same preparation) introduces the greatest error when sections with many fat granules are estimated.

The reproducibility of the results obtained by the semiautomatic method of assessment is very high. Only in 20% of cases did repeated measurements show differences in indicator readings of more than 10% of the initial values. The method of semiautomatic assessment of the degree of fatty degeneration can therefore be recommended for use in morphometric research practice provided that the reservations mentioned above are taken into account.

The method of semiautomatic assessment of the degree of fatty degeneration on the TAS instrument (Leitz-Wetzlar, West Germany), developed and tested by the writers, can be recommended for morphologic research practice.

LITERATURE CITED

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