THE EFFECT OF CARBON DISULPHIDE ON THE METABOLISM OF PYRIDINE NUCLEOTIDES AND NICOTINAMIDE

Teresa Wronska-Nofer, **B.CH.**

Institute of Occupational Medicine in the
Textile and Chemical Industries
Teresy 8, Lodz, Poland

The toxic action of carbon disulphide is many sided. For example, it has been known, that, depending on the severity of exposure, the prevailing symptoms may be the result of a neurotoxic action (at higher concentrations) or a vasculotropic one (at lower concentrations). Similarly, various known biochemical disturbances in the organism may be caused by this compound. At present, it seems difficult to answer the question whether the different disturbances appear independently each from the other or as a center of initial action with derivative symptoms which are being actually observed.

Our present studies on the metabolism of nicotinic acid in the course of carbon disulphide intoxications were started on the basis of the following earlier observations:

1. Exposure to CS_2 brings about disturbances in the urinary level of the nicotinic acid, manifested by an increase of the N^1-methylnicotinamide. That was first observed by Liniecki (1) and later confirmed by Wrońska et al. (2) to occur in rats. However, similar disturbances were also found in workers exposed to CS_2, in the viscose rayon industry (3). Moreover, some quantitative relationship was observed between the elevation of N^1-methylnicotinamide and the actual concentration of CS_2 in the air (Fig. 1), (4). It was found in rats exposed to a concentration of 0.9 mg/l of CS_2 that increase of N^1-methylnicotinamide in the urine takes place already at the beginning of chronic exposure (after 2 days), reaching a steady state of the elevated level after one week of exposure **(Figure 2) (4).**

2. Some biological importance of the above disturbances seem evident if one considers the influence of nicotinic acid on the pathways of the lipid metabolism: Chronic exposure to CS_2 results in changes of the lipid metabolism consisting of an increase of the cholesterol, phospholipids and triglycerides in blood. These changes could be prevented by feeding of the animals with nicotinic acid (5).

Collaborating Research Staff: J.A. Sokal, M.Sc., S. Tarkowski, D.Sc., R. Górny, M.Sc. (Department of Biochemistry) and **Dr. Habil. S. Szendzikowski,** M.D., J. Stetkiewicz, B.M. (Department of Pathomorphology).

The biochemical background of the above phenomenon is still obscure. With respect to the changes in the metabolism of nicotinic acid it seemed to be interesting to find out the sources of the excess of nicotinic acid appearing in the urine in form of the N^1-methylnicotinamide, if constant uptake of nicotinic acid from the food is accepted.

The existing data point to the fact that the bulk of the systemic nicotinamide is present in the body in the form of nicotinamide adenine nucleotides. Therefore, theoretically at least, the only source for the elevated levels of nicotinic acid could be the pool of the nucleotides; carbon disulphide could, eventually, increase their degradation rate, resulting in an increased excretion of the nicotinamide (as a methylated derivative or nicotinic acid). If this hypothesis is taken as a basis, two possibilities seem to have existed: (a) the increased degradation rate of nucleotides has been accompanied by similar increase in the rate of synthesis (increased total turn over rate), allowing the actual absolute level of nucleotides to remain unchanged; (b) otherwise, when there was no increase in the synthesis of nucleotides, the elevated excretion would result in a depletion of the systemic levels of the nucleotides if the exposure to CS_2 had persisted for a sufficiently long time.

In the present studies attempts were undertaken to answer the following questions:

a. Whether, apart from disturbance of N^1-methylnicotinamide, also the excretion of other metabolites of nicotinic acid is disturbed by exposure to CS_2;

b. Whether the elevated excretion of the nicotinic acid metabolites is reflected by a drop in the level of systemic nucleotides, and

c. What is the biochemical background of the disturbances in question. Attempts to approach the above problems are given below in detail.

I. The nicotinamide metabolites in the urine of rats exposed to CS_2.

The underlying data on the excretion of the metabolites of nicotinic acid were fragmentary only and were based entirely on the fluorimetric determinations. Therefore, we decided to check once again the accepted assumption using methods which differ from those applied previously and which allow the estimation of N^1-methylnicotinamide as well as nicotinic acid and nicotinamide.

The evaluation of the excretion of the nicotinic acid metabolites has now been based on: (a) quantitative determination of the metabolites

by chemical methods, and (b) on the measurement of activity of individual metabolites, following administration to rats in vivo of nicotinic acid labelled with ^{14}C in carboxyl group. This was done in separate experiments in the following way:

1. The rats exposed to CS_2 and the controls were placed in metabolic cages and in the daily urine collected the following metabolites were determined: (a) N^1-methylnicotinamide (fluorimetrically after Huff et al.(20)); the "total nicotinic acid" colorimetrically and the nicotinamide, both after Wrońska et al. (21,22). The content of the free nicotinic acid was calculated from difference between "total nicotinic acid" and nicotinamide.

2. In the other series of experiment, rats (exposed and controls) were given intraperitoneally nicotinic acid or nicotinamide (2 µmoles of total activity 20 µC per rat) and the daily volume of urine was collected. The metabolites of nicotinic acid were separated by electrophoresis (23) or paper chromatography in the system 80% n-propanol or n-butanol: aceton: water (9:1:10). The measurement of the activity was done on the Nuclear-Chicago flow-counter.

The results presented in Table 1, confirm our view on the increased excretion of the nicotinic acid metabolites in exposed rats. N^1-methylnicotinamide was increased by 126%, the nicotinic acid by 34%, only the nicotinamide was not affected. The same trend is evident also from the isotopic studies (Table 2).

The new element of this study is that the discussed disturbances are not limited to elevated excretion of N^1-methylnicotinamide but also concern the second metabolic product of nicotinamide, namely nicotinic acid itself.

II. Studies on the levels of the nicotinamide-adenine dinucleotides in the tissues of rats exposed to CS_2.

A. Methods of determination of the nicotinamide-adenine nucleotides in the tissues.

The methods most often recommended for the quantitative determination of the nicotinamide-adenine dinucleotides include enzymatic procedures which are based on preparations that are hardly available in Poland, and also very expensive. The study therefore, had to be started on the development of simple modifications in which the above obstacle would be eliminated. Consequently, these attempts had to be extended to cover the specific demands for analysis of different kinds of tissues which were to be investigated.

The basic principle underlying the modifications was that of fluorimetric procedure described by Lowry et al. (6) and Bassham et al. (7). It appeared necessary to use a somewhat different procedure of determination when performed on the liver, blood and muscles than on the brain.

Liver, blood, and muscles*: The elaborated procedure (Figure 3) consists of 3 basic stages:

1. Selective extraction from the tissues of the reduced and oxidized forms, respectively, using conditions differing principally in pH and temperature: hot 0.1 sodium carbonate and cold 5% TCA.**

2. The separation of the phosphorylated forms was performed with the oxidized forms only: therefore, the reduced ones had to be first oxidized, and this was achieved by using phenazonium methosulphate. From the obtained NAD and NADP solutions the former was reduced in the system containing ethanol and alcohol dehydrogenase. In this way the two compounds differing initially by the degree of phosphorylation were converted into those differing in the redox state and as such could be separated using the initial procedure of acidic - alkaline destruction of one component.

3. The fluorescence was measured after proper incubation in 6 N NaOH at 37°C., 60 min, using "Farrand" fluorimeter (Model A), primary filter: 5860, and the secondary filters: No. 4308 and 3389 or "Opton" spectrofluorimeter (Model PMQ-II - excitation 370 nm and transmission 460 nm).

The recovery of standards (NAD, NADP and NADH) added to the tissue was almost complete and the standard error was ± 10%. The sensitivity was about 50 μmoles/g of liver.

The low content of the nucleotides, and especially of NADP in blood and muscles, was the cause for which in these tissues the procedure had to be limited to the determination of sums of the oxidized (NAD+NADP) and reduced forms (NADH+NADPH). The recovery of standards was satisfactory also with these tissues (for NAD-95%).

Brain***:In the way described above the content of the nicotinamide adenine dinucleotides may be also determined in fresh brain tissue. It has been shown, however, that the post-mortem changes of the

* For details see: Sokal et al. (13); Sokal and Wrońska-Nofer (12).

** TCA - trichloroacetic acid.

*** Sokal - in preparation.

content (Figure 4), and specially of the redox state of the nucleotides, are very rapid resulting in values that are erroneous with respect to the state existing in vivo. On the other hand, if the described procedure was applied to frozen brain tissue, artifact took place resulting in lowering of the levels of the reduced forms. Therefore, the extraction procedure had to be modified once again by introducing the basic principle of Burch et al., (8). From the latter the presence of an antioxidant (cysteine) and a low temperature (0°) appeared to be essential to counteract the oxidation of the reduced forms.

Using the methods presented above the levels of nicotinamide-adenine dinucleotides were determined in animal tissues, and the obtained levels are presented in Table 3.

B. The influence of the prolonged exposure to CS_2 on the levels of nicotinamide-adenine nucleotides in the tissues of rat*.

These studies were performed in order to answer the question whether the increased excretion of the nicotinic-acid metabolites occurs at the expense of the systemic pool of nucleotides. As mentioned formerly, this seemed probable since the nicotinamide-adenine dinucleotides represent the main pool of the systemic nicotinamide (for instance in the liver, about 80%; see Ricci and Pallini (9)). On the other hand, prolonged elevated excretion of the nicotinic acid metabolites in a degree found experimentally if kept over sufficiently long time-period, would consume an excess of nicotinamide in the amount comparable with their content in the whole body of the rat.

The experiment was kept over 5 months on rats exposed daily to CS_2 in concentration of 1.8-2 mg/l. The levels of the nucleotides were determined in groups of animals sacrificed after different time-periods of exposure, and the determinations were performed on blood, liver and muscles; these tissues contain jointly about 80-90% of the systemic pool of nucleotides (10). White female rats of the Wistar strain were used. The animals were fed standard ISM diet.

The results are shown in Tables 4 and 5. From Table 3 it follows that within the period of 5 months only subtle changes in the levels of nucleotides could be found in the tissues; in liver a slight increase occurred both of the reduced and oxidized form, resulting chiefly in the form of an increase of NAD and NADPH levels (Table 5). The reverse

* For details see Wrońska-Nofer et al. (10); Sokal (24); part of the data are in preparation.

was found in blood and muscles. The above changes were reached, after 5 months duration of exposure, about 20% different from the controls. The drop of the oxidized form of nucleotides in muscles was lower by about 9%.

The above had to be compared with the predictions resulting from the increased output of nicotinamide metabolites. The latter have not been determined parallely in this experiment but the data have been taken from the previous experiments as sufficient for such comparisons. From those it could be calculated that the excess excretion of nicotinic acid metabolites amounted in rat to 0.38 µmole/day (2,14). If such a daily excess is summed up with time and the result is substracted from the whole systemic pool of nicotinamide-adenine nucleotides, the predicted drop of their systemic level is obtained (Figure 5). After 5 months of exposure, the latter should drop to only 25% of the initial (control) value. The actual decrease, however, has not exceeded some 15%, a value far from that resulting from the predicted hypothesis. It seems justified, therefore, to exclude the assumption that the increased output of nicotinamide metabolites in rats exposed to CS_2 occurs at the expense of the systemic levels of nucleotides.

The above does not apply to later periods of exposure, when severe injury is already observed (limb paralysis, drop of body weight and especially of the muscles). These severe symptoms are accompanied also by some changes of the nucleotides in the tissues.

The results of experiments with such a prolonged exposure given in Table 6, show that at that time there are still no essential changes in the level of nucleotides in the brain. In the muscles, however, there is an essential drop of the total level of nucleotides reaching about 50%, after 8 months of exposure. In this type of a very prolonged exposure, apart from the total tissue levels, also the distribution among subcellular fractions of liver was determined. Whereas, total deviations found in the liver are not very pronounced, of some interest may be the drop of the nucleotides in the mitochondrial fraction (Table 7). This may be connected with some damage to these subcellular elements as found by Woyke (15).

III. The turnover of the nicotinamide-adenine nucleotides in the liver of rats exposed to CS_2.

From the former data it follows that the exposure to CS_2, if limited to the time period of half a year, does not influence considerably the levels of the nicotinamide-adenine nucleotides in the tissues. Therefore, it had to be expected that increased excretion of N^1-methylonicotinamide is accompanied not only by the higher degradation rate of the nucleotides but that the whole turnover rate is accelerated.

It is known that into the nicotinamide-adenine nucleotides are incorporated both, nicotinic acid and nicotinamide, the latter after previous desamidization. The studies on both these processes are presented below.

A. The incorporation of nicotinic-acid into the nicotinamide adenine nucleotides.

The experiment was performed on 29 rats (in this number 15 controls). The rats were given intraperitoneally 2 μmoles of nicotinic acid labeled ^{14}C (20 μC). The rats were killed 15, 30, 60, and 240 minutes after injection of nicotinic acid. Immediately after sacrification, the oxidized forms of nucleotides were extracted from the liver with cold 5% TCA and precipitated with acetone. The precipitates were further dissolved in water* and the two forms, NAD and NADP, were separated by column chromatography (column filled with Dowex 1x8, 200-400 mesh) using continuous concentration gradient of formic acid**. The final determinations were done fluorimetrically (as described formerly); the radioactive NAD, collected in the effluent of the column, was applied on the planchettes and the measurement of B-radiation was done on the Nuclear-Chicago Flow-Counter.

The results are presented in the Figure 6. The specific activity of NAD rose within 30 minutes after injection in both groups and later it decreased. However, the dynamics seemed to differ essentially in both compared groups. In rats exposed to CS_2 both the rise of specific activity in the first period and the drop in later stage were more rapid than in controls. This confirms our assumption of an increase of the total turnover rate of the nucleotides in carbon disulphide exposure.

B. Deamidization of the nicotinamide.

To assess the changes in the process of deamidization of nicotinamide its activity was measured in vitro in the liver homogenates. The activity of the nicotinamide amidohydrolase was measured using the method of Kirchner et al. (18) with ^{14}C labeled nicotinamide as substrate. This method is based on the determination of nicotinic acid liberated during incubation at 37°C in medium consisted of liver homogenate in 0.033 M phosphate buffer, pH 8.6 and labeled ^{14}C nicotinamide. The results were presented in μmoles of nicotinic acid per mg of protein of the homogenate, per hour*** This experiment was performed on 19 rats (in this number 9 control rats).

* This stage according to Gordon (16). In control experiments practically complete recovery (over 95%) of the oxidized nucleotides from the acid extract was obtained by acetone precipitation.

** This procedure after Hurlbert et al. (17). In control experiment almost complete recovery (ab. 90%) was found of the exogenous nucleotides added to the acetone precipitate.

*** The protein content of homogenate after Lowry et al. (19) using fraction of the bovine albumin as a standard.

The results listed in Table 8 point to an increase of activity of the nicotinamide-amidohydrolase in exposed rats by about 60%. This increase seems to act in favor of the accelerated turnover rate of the nucleotides giving more direct substrate for the incorporation process of nicotinic acid. On the other hand, this trend can hardly be explained as being in favor of the increased methylation of nicotinamide. In this way, a new gap arose in our hypothesis, where the increased excretion of N^1-methylnicotinamide was accepted as a basic assumption.

Conclusions

The chief aim of the present study was to elucidate the mechanism by which carbon disulphide influences the increase of urinary excretion of N^1-methylnicotinamide. The present data allow the following conclusions to be drawn:

a. The exposure to carbon disulphide results in elevated urinary excretion not only of N^1-methylnicotinamide but also of nicotinic acid and of the total pool of its metabolites.

b. The above increase of the urinary excretion of the nicotinamide metabolites does not occur at the cost of the systemic pool of the nicotinamide-adenine dinucleotides.

c. The increased rate of incorporation of nicotinic acid into nucleotides, as well as increased rate of deamidization of nicotinamide points to the fact that the elevated excretion of nicotinamide metabolites reflects an increase, often whole turnover rate, of the nicotinamide-adenine dinucleotides in CS_2 exposure. Whatever the mechanism of this phenomenon, it is responsible for keeping the pool of nucleotides on unchanged level.

d. The data do not allow, yet, to draw any final conclusions as to the mechanism of the duscussed phenomena. Obviously further studies would be necessary including those of the systemic levels of the different forms of nicotinic acid. Also, there are no data on the methylation process itself, as well as on the level of the donors of the methyl-groups. The basic question which remains to be answered, however, is that of sources of excess nicotinamide for its increased turnover. While the assumption of increased synthesis via tryptophan seems most probable it still remains to be proved.

e. It is not clear whether the discussed disturbances in the metabolism of nicotinamide represent a phenomenon specifically conditioned upon the exposure to CS_2 or not. The recent reports on similar disturbances occuring in animals exposed to X-rays let us suspect that the mechanism in question may be entirely nonspecific. This however, needs further detailed study.

IV. The evolution of morphological changes in rats in the course of prolonged exposure to carbon disulphide.

Histopathological studies in rats intoxicated with CS_2 have been undertaken independently of the biochemical one which aimed at explanation of the mechanism of disturbances in the metabolism of nicotinic acid in CS_2 intoxication.

In rats exposed for 8-10 months to CS_2 vapours, onset of the paresis of the hind limbs of a various degree developing into paralysis and muscle atrophy was observed. In the atrophied muscles a reduction of the level of nicotinamide adenine nucleotides approximately to one half of control values, was detected. With an anticipation to trace the relationship between the biochemical disturbances and the grade of structural lesions induced by CS_2, separate experiments were undertaken. It was aimed as well at following the process of evolution of the histopathological changes in muscles, peripheral nerves and central nervous system, as at determining in parallel concentration of the nucleotides muscle tissue in the course of maintained exposure to carbon disulphide. White rats of Wistar strain, controls and exposed to CS_2 at concentration of 1.5-1.8 mg/l over the period of 2-14 months, were used in this study.

For investigations of the muscles, quadriceps femoris, gastrocnemius and tibialis anterior were chosen. The material suitable to biochemical procedures was dissected from the femoral quadriceps at the moment when sacrifice of each animal was just accomplished. Wet tissue weight of the muscles (excluding quadriceps) was recorded after dissection. The muscles were then cut into transverse and longitudinal pieces for further histological and morphometrical assay. Hematoxylin and eosin and PTAH (Mallory's phosphotungstic-acid hematoxylin) stains and the McManus periodic-acid-Schiff (paS) procedure were employed using paraffin sections.

In the morphometric estimations transverse sections of gastrocnemius were used. The diameter of individual muscle fibers was recorded at random, the obtained values divided into eight classes in the range between 20-100 μ of thickness and the percent distribution expressed in the form of histograms.

From the general observations in this group of experimental animals first signs of muscular weakness were noted within the seventh month of the intoxication. Two months later paresis of hind extremities was sufficiently evident developing into paralysis at the fourteenth month of exposure.

For the comparative analysis wet muscle weight was expressed in direct values (absolute weight) and in relation to the body weight (relative weight).

Over the whole period of 14 months the weight of gastrocnemius and anterior tibial muscle of the intoxicated animals was constantly lower than the controls (Figure 7). Through the fifth month of exposure the difference was due to the retardation of muscle growth under the influence of carbon disulphide, and it was seen in the absolute as well as in the relative values. Later the muscle weight (both absolute and relative) in controls remained unaltered, whereas in the experimental group its gradual fall was observed. The latter was very slow in the fifth month, it became faster in the seventh, to become particularly pronounced within the 12-14 month of the exposure.

In the microscopic picture the presence of atrophied muscles fibers was a constant finding from the sixth month up to the final stages of the experiment. The grade of atrophy is nonuniform in various areas of the same sections. Dispersed across the whole thickness of the muscle are small groups of thin slightly basophilic muscle fibers (Figure 9). Their borders are indistinct and the number of nuclei is excessive. Within some of the bundles solitary hypertrophied fibers are represented (Figure 10). In the remaining muscle except for a slight hypertrophy no apparent morphological lesions can be seen.

Although the typical signs of atrophy were absent from the histological picture of muscles prior to the sixth month of exposure, some quantitative changes in the diameter of muscle fibers were detected by means of the morphometric procedures. They can be seen as a shift toward the extreme classes in the histogram of the gastrocnemius muscle of rats exposed for 5 months to carbon disulfide (Figure 8). The shift represents a simultaneous increase in the number of both the thinnest and the thickest (presumably hypertrophic) muscle fibers. In the following months, when the typical signs of muscle atrophy appear in the histological picture, a reliable count of thin fibers can be done, but the shift to large diameters is still evident.

From the nervous system the brain, cerebellum, brain stem, spinal cord and two peripheral nerves, ischiadic and femoral, were subject to histological study. Routine methods of fixation, embedding and staining, the latter consisting of hematoxylin and eosin, PTAH and the Luxol Fast Blue stain with cresil violet counterstanding (36) were employed in serial sections. In addition portions of the ischiadic

and femoral nerve fixed in 2% glutaraldehyde were impregnated subsequently in osmium tetroxide, stained with Pal-Kulczycki's hematoxylin and embedded in Epon 812. From this material sections 1 u thick were cut on an ultramicrotome for the ordinary microscopic examinations.

In the neuronal cells of the central nervous system no apparent lesions were encountered. From all regions of white matter subjected to investigation porosis of a small degree was noted in the anterior and lateral funiculi of the spinal cord of the experimental rats sacrificed after 12 and 14 months of exposure. Within the somewhat spongy structures a few globular or polyglonal hyaline clumps could be found. They corresponded, in all probability, to the swollen axis-cylinders.

No definite conclusions about the actual condition of the peripheral nervous fibres could be drawn from the tissues prepared according to the routine procedures, whereas in the semi-thin sections after osmium impregnation signs of degeneration in separate fibres were detected as early as 4 months after beginning of exposure. With the prolonged intoxication very distinct alterations in the diameter of individual fibres, more abundant interstitium and thickening of the walls of intraneural blood vessels were also observed (Figures 11, 12). Mylein sheets were spared in the majority of the fibres, with occasional small defects or partial lamination. Complete loss of mylein sheet was apparent in a small percentage of fibers, evidently due to their total degeneration (Figure 12).

Investigations of the level of nucleotides in the femoral quadriceps muscle were conducted from the 1-12 months of exposure. Determinations were performed according to the fluorimetric method of Sokal et al. (8).

Seven months of exposure to carbon disulphide remained without an appreciable effect on the concentration of nucleotides in the muscles; during the following months its slight decrease was recorded. A considerable loss of the muscle nucleotides occured in the final stages starting at the end of 10 months of carbon disulphide intoxication (Table 9).

Conclusions

It seems that results of our investigations do justify the conclusion that muscular changes do belong to the syndrome of chronic carbon disulphide poisoning, produced under the experimental conditions described above.

The proof seems to lie in the presence of lesions of peripheral nervous system and in the fascicular character of muscle atrophy (32,34,38). Prior to appearance of the latter the morphometric procedure was effective in showing that initial stage of muscle atrophy was represented by characteristic quantitative deviations in fiber diameters. The important part of these changes could be characterized as hypertrophy of some fibers parallel to initial atrophy of the others. Furthermore, no appreciable morphological alterations were detected that would be suggestive of any diffuse muscle lesion pointing to a direct influence of CS_2 or its metabolites on the muscle fibers.

It should be emphasized that a prolonged exposure to carbon disulphide was necessary to produce a substantial decrease in the amount of nicotinamide-adenine nucleotides in the muscles.

At that stage of intoxication the fall of muscle weight amounted to 50% of the control values and the histological evidence of atrophy was already unequivocal. The changes in the level of nucleotides should be accepted therefore as an effect of neurogenic atrophy, due to the partial denervation of the muscle in chronic carbon disulphide poisoning.

Development of the syndrome of chronic intoxication was slow although a fairly high concentration of CS_2 was employed. The histological changes in the central nervous system were very slight as compared with the lesions produced by CS_2 in dogs (33) and rabbits under the conditions of experimental intoxication (35,37).

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Table 1

The Content of Nicotinic Acid Metabolites in the Urine of Rats Exposed to CS_2*

Group of Animals	Number of animals	Nicotinic Acid		Nicotinamide		Total nicotinic Acid		N^1-methylonicotinamide	
		µg	%	µg	%	µg	%	µg	%
Control	13	29.8±9.9	100	30±9.9	100	59±10.9	100	157+71	100
CS_2	13	40±13.6**	134	32.5±7.05	108	72+13.7**	122	355+172	226

* The values given in the Table present µg of metabolites excreted in the 24 hours urine ± S.D.

** Difference statistically significant as compared with control: $P < 0.05$.

Table 2

Activity of Nicotinic Acid Metabolites in the Urine of Rats.

Group of Animals	Nicotinic Acid		Nicotinamide		N^1-Methylnicotinamide	
	C.P.M. x 10^{-4}	%	C.P.M. x 10^{-4}	%	C.P.M. x 10^{-4}	%
Control	13.06	100	2.62	100	13.05	100
CS_2	14.93	114	2.25	86	38.19	292

The values given in the Table present activity of nicotinic acid and its metabolites excreted in the daily urine of rats 24 hours after intraperitoneal injection of 20 uC of nicotinic acid (carboxyl -C^{14}).

Table 2A

Activity of N^1-Methylnicotinamide in the Urine of Rats.

Injected Compound	Group of Animals	N^1-Methylnicotinamide		
		C.P.M. x 10^{-4}	% Excreted Dose	%
Nicotinamide-7-C^{14}	Control	13.04	17.7	100
	CS_2	30.5	34.8	235
Nicotinic Acid-7-C^{14}	Controls	7.35	10.2	100
	CS_2	14.02	20.6	191

The values given in the Table present activity of N^1-Methylnicotinamide excreted in the daily urine of rats 24 hours after intraperitoneal injection of 20 μC of nicotinamide (carbonyl-C^{14}) and nicotinic acid (carboxyl-C^{14}).

Table 3

The Level of the Nicotinamide-Adenine Dinucleotides in the Rat Tissues.*

Liver				
Authors	NAD	NADH	NADP	NADPH
Authors own data	447 ±40	199 ±38	96 ±15	409 ±56
Glock, et al. (28)	654	178	34	308
Lowry, et al. (27)	628	252	115	502
Bassham, et al. (7)	485	133	25	251
Sundarm, et al. (25)	496	119	41	269
Ville (26)	390	170	15	350
Pande, et al. (29)	300	82	79	254

Brain		
Authors	NAD + NADP	NADH + NADPH
Authors own data	406 ±31	61 ±6
Glock, et al. (28)	200	144
Lowry, et al. (27)	329	118
Garcia-Bunuel et al. (30)	309	58
Lowry, et al. (6)	251	119

Blood		
Authors	NAD + NADP	NADH + NADPH
Authors own data	112 ±11	55 ±9
Glock, et al. (28)	90	58
Lowry, et al. (27)	124	99
Cartier, (31)	122	110

* The levels of nucleotides in blood presented in μmoles/ml of total blood, in the brain and in the liver in μmoles/g of wet tissues. ±S.D.

Table 4

Changes in the Nicotinamide-Adenine Nucleotides Levels in Blood, Liver and Muscles of Rats in the Course of Exposure to CS_2.*

Duration of Exposure (Months)	Blood		NAD + NADP Liver		Muscles	
	Control	CS_2	Control	CS_2	Control	CS_2
1	111±11	104±14	528±51	563±50	535±48	529±57
2	108± 4	109± 9	589±81	603±59	542±28	525±50
3	115±13	96±11	555±35	642±40	573±24	530±32
5	119±10	100± 4	525±53	627±57	582±35	527±35
			$NADH_2$ + $NADPH_2$			
1	48± 9	47±13	597±150	514±98	98±12	86±12
2	59±11	47±13	591±116	603±91	93±10	93±18
3	56± 5	43± 6	596±66	669±56	92±18	98±13
5	56± 7	46± 3	605±104	730±179	60± 6	70±11

* The level of the nicotinamide nucleotides in blood presented in μmoles per 1 ml of whole blood, in the liver and in muscles in μmoles per 1 g of wet tissue +S.D. Mean values from 7-10 animals are given.

Table 5

The Level of the Nicotinamide-Adenine Dinucleotides in the Liver of Rats After 5 Months of Exposure to CS_2*

Groups of Animals	Number of Rats	Weight of Liver (g±S.D.)	NAD	NADP	$NADH_2$	$NADPH_2$
CS_2	7	7.0±1.3	506±48**	95±18	228±84	502±117
Control	9	6.8±0.7	439±50	86±14	185±35	409±59

* The level of the nicotinamide neucleotides presented in μmoles per 1 g of wet tissues ± S.D.

** Difference statistically significant as compared with control: $p < 0.05$.

Table 6

The Levels of Nicotinamide-Adenine Dinucleotides in Brain, Liver, and Muscles of Rats After Long-Term Exposure to CS_2

Group	Brain		Liver		Muscles	
	NAD+NADP	NADH+NADPH	NAD+NADP	NADH+NADPH	NAD+NADP	NADH+NADPH
CS_2	385±23	62±5	623±26	456±62**	310±44.2**	74±15**
Control	396±10	58±3	629±62	523±27	613±40.4	109±23.3

* The levels of nucleotides presented in μmoles per 1 g of wet tissues ± S.D.

** Difference statistically significant as compared with control: $p < 0.05$.
Mean values from 10 - 14 animals.

Table 7

Intracellular Distribution of Nicotinamide Adenine Dinucleotides in the Liver of Rats Exposed to CS_2*

Cellular Fraction	Group	Total Protein	NAD+NADP	$NADH_2$+$NADPH_2$
Homogenate	CS_2	201±9	623±26	456±62**
	Control	204±8	629±62	523±27
Nuclear Fraction and Debris	CS_2	36±5	43±7	50±15**
	Control	34±8	49±7	67±15
Mitochondrial Fraction	CS_2	48±3	115±18	203±37**
	Control	48±3	124±20	254±24
Microsomal Fraction	CS_2	33±3	49±4	29±4
	Control	31±3	49±11	27±8
Soluble Fraction	CS_2	87±7	410±17	221±25
	Control	88±7	415±54	199±18

* The levels of proteins in mg and nucleotides in µmoles are expressed per 1 g wet liver tissue ± S.D. Mean values from seven animals.

** Difference statistically significant in relation to control: $p < 0.05$.

Table 8

Nicotinamide Deamidase Activity in the Liver of Rats Intoxicated with CS_2*

Group of Animals	Number of	Activity	%
Control	9	16.6±2.1	100
CS_2	10	27±1.7**	163

* Enzyme activity expressed in μmoles of nicotinic acid formed from nicotinamide during one hour incubation at 37° calculated for 1 mg of protein of liver homogenate ± S.D. The incubation medium was composed of: 1 ml of 10% liver homogenate in 0.033 M phosphate buffer, pH 8.6 containing 200 μmoles of ^{14}C-nicotinamide of total activity 1 μC.

** Difference statistically significant, $p < 0.01$.

Table 9

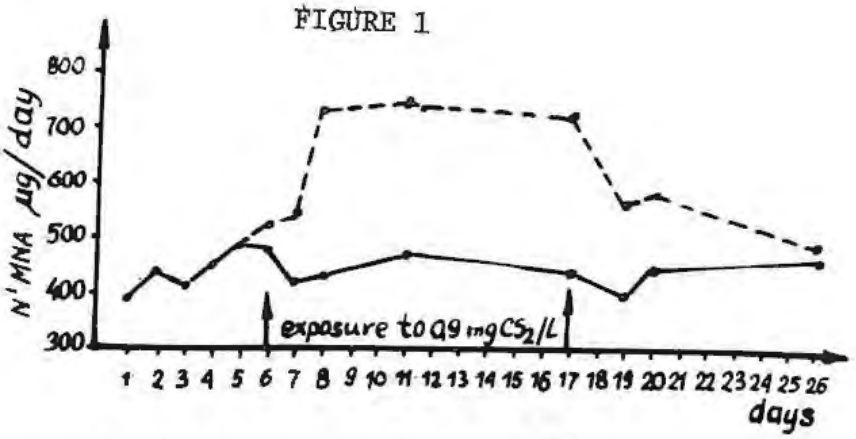
Change in the Nicotinamide-Adenine Nucleotides Levels in Muscles of Rats in the Course of Exposure to CS_2*

Duration of Exposure (months)	Number of Animals	NAD+NADP		$NADH_2+NADPH_2$	
		Control	CS_2	Control	CS_2
1	6	535±48	529±57	98.5±21	85±12
2	8	526±53	527±50	131±14	133±26
5	6	533±30	562±72	94±10	92.5±11
7	6	577±82	569±58	94±19	89±16
10	7	574±29	472±38**	118±16	97±21
12	5	573±36	400±52**	119±19	87±14**

* The levels of nicotinamide nucleotides in muscles presented in μmoles per 1 g of wet tissue ± S.D.

** Difference statistically significant as compared with control: $p < 0.05$.

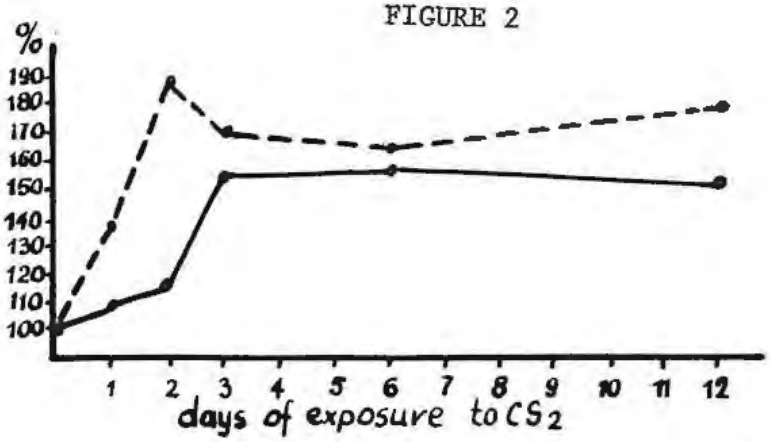
FIGURE 1



The daily excretion of N^1-methylnicotinamide in the urine of rats

——control group ; ----- exposed to CS_2.

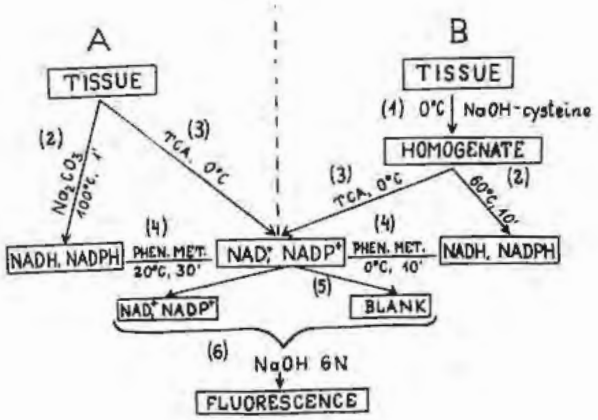
FIGURE 2



Relationship of CS_2 concentration and N^1-methylnicotinamide daily excretion in the urine of rats exposed to CS_2.

——— 0.9 mg CS_2/l ; ------ 2.0 mg CS_2/l.

FIGURE 3



Scheme of the procedures for determination of the nicotinamide-adenine dinucleotides.

A- Procedure for fresh liver tissue/Sokal et al,1969/

B- Procedure for frozen brain tissue.

1- Tissue homogenization in cold NaOH-cysteine solution

2- Alkaline extraction of reduced nucleotides

3- Acidic extraction of oxidized nucleotides

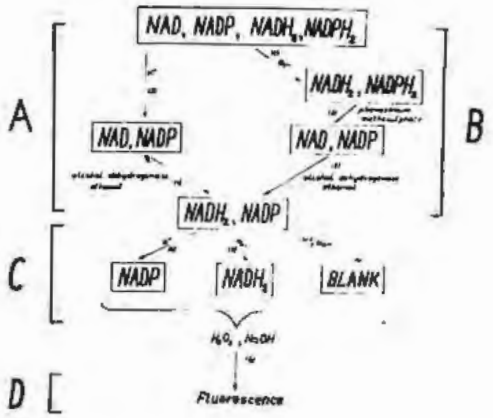
4- Oxidation of reduced nucleotides with phenazonium methosulphide

5- Alkaline destruction of nucleotides

6- Development and measurment of fluorescence

Abreviations: TCA-trichloroacetic acid,
Phen.met- phenazine methosulphate.

FIGURE 3a.



Scheme of the procedure.

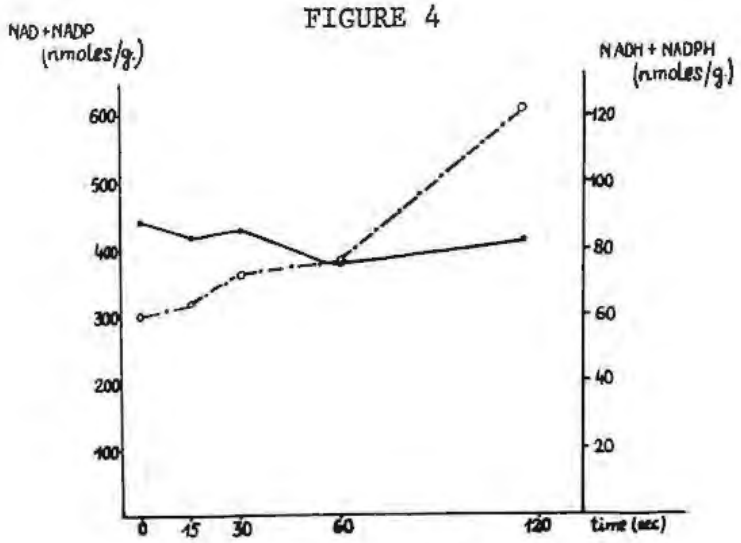
A - acidic extraction and enzymic reduction;

B - alkaline extraction, oxidation with phenazon methosulphate and enzymic reduction;

C - separation of $NADH_2$ and NADP;

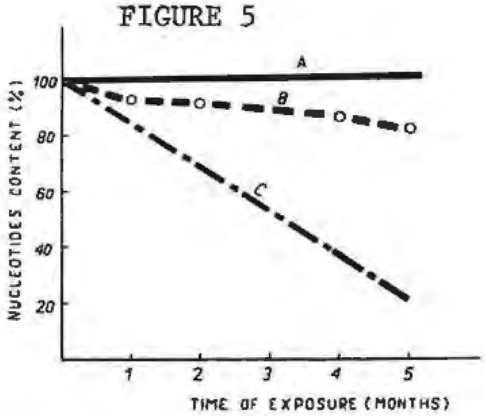
D - development and measurement of fluorescence

FIGURE 4



The effect of duration of ischemia upon the levels of nicotinamide-adenine dinucleotides in the rat brain tissue (—— NAD+NADP; —·—·— NADH+NADPH)

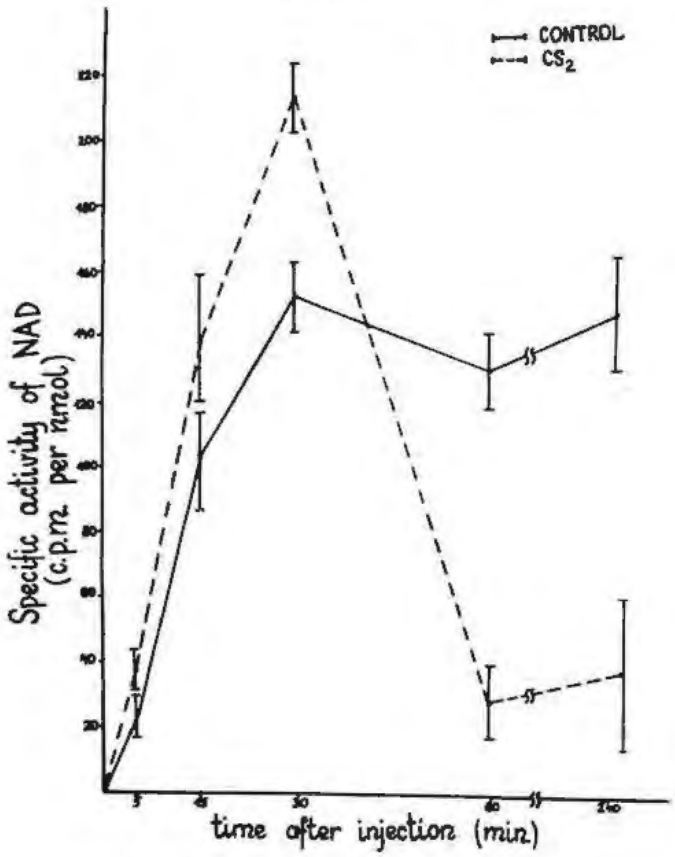
FIGURE 5



Changes in the level of the nicotinamide-adenine nucleotides in the whole body of rats in the course of 5 months exposure to CS_2.

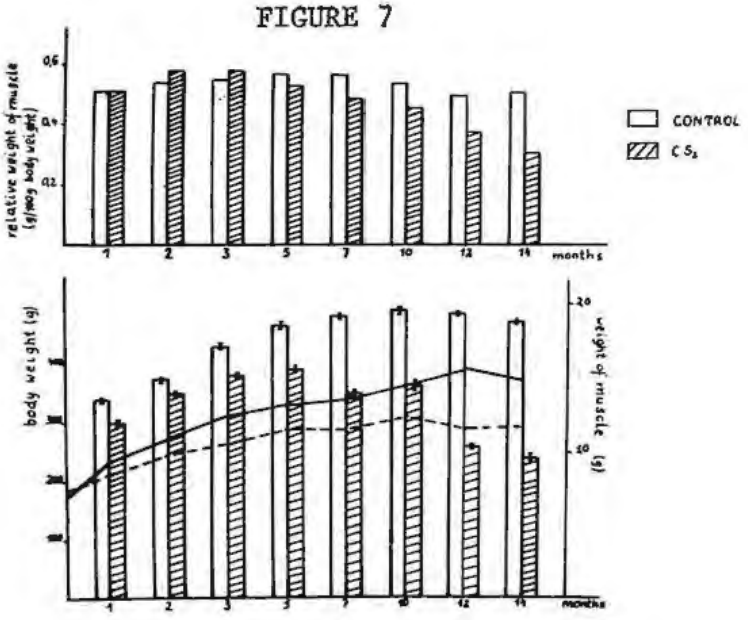
A - control; B - experimental data in rats exposed to CS_2; C - theoretical drop resulting from increased elimination of nicotinamide metabolites.

FIGURE 6



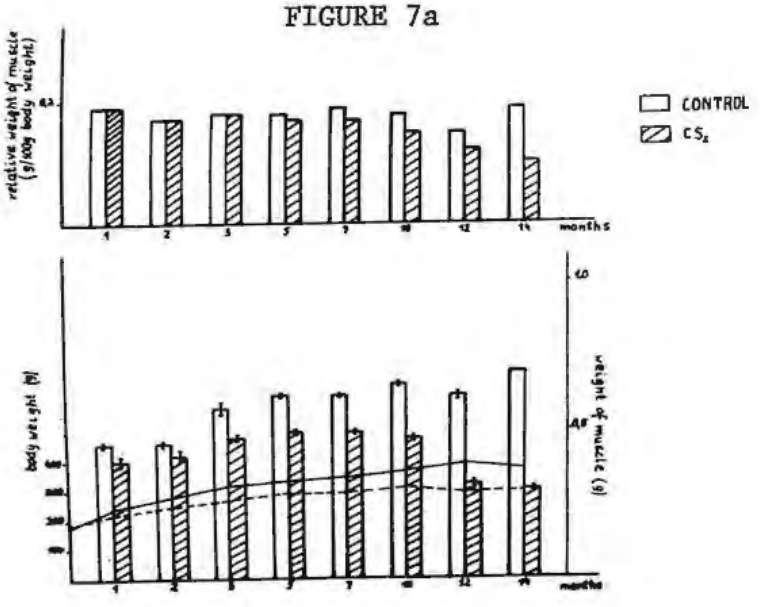
Dynamics of incorporation of nicotinic acid into NAD molecule in the liver of rats after intraperitoneal injection of 2 μmoles of ^{14}C-nicotinic acid (total activity 20 μC)

FIGURE 7



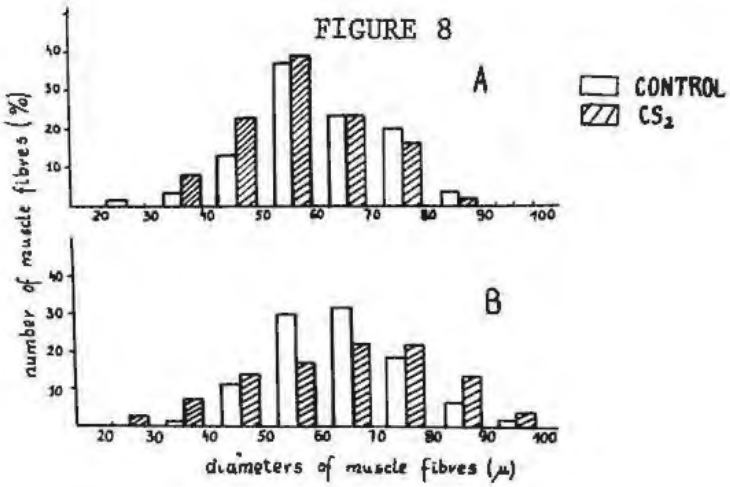
The body weight and weight of gastrocnemius muscle in rats during exposure to CS_2.

FIGURE 7a



The body weight and weight of tibialis anterior muscle in rats during exposure to CS_2.

FIGURE 8



Histogram of the diameters of muscle fibres from the gastrocnemious muscle of the control and exposed rats

A - 3 months of exposure to CS_2
B - 5 months of exposure to CS_2

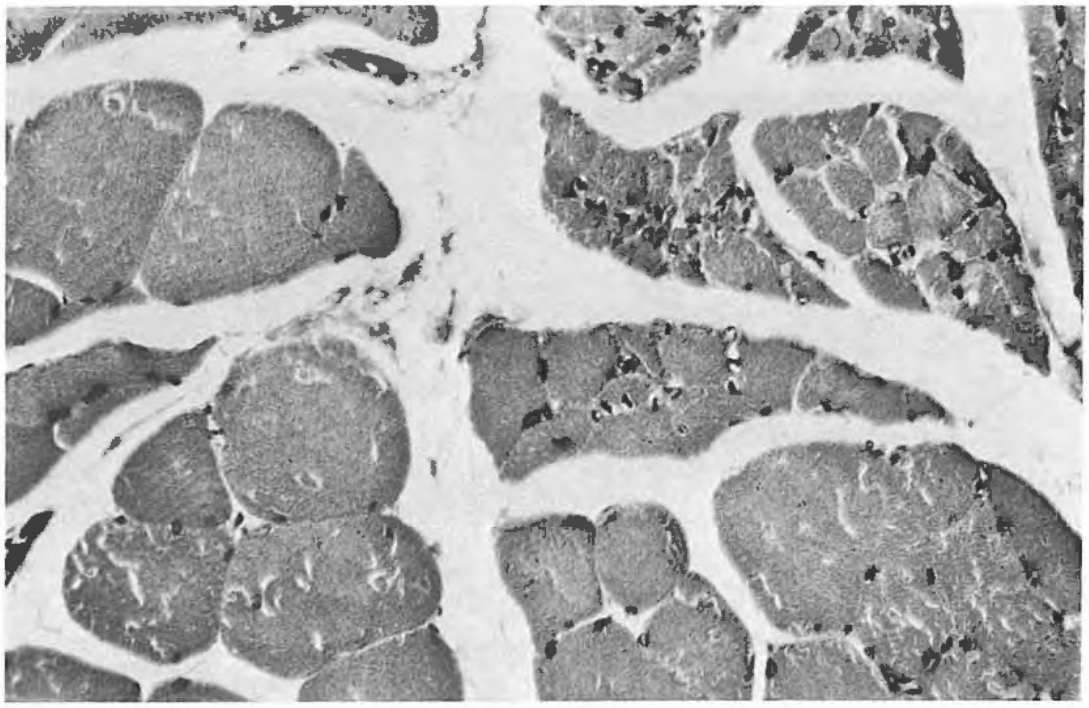


Figure 9. A bundle of muscle fibers undergoing atrophy is surrounded groups of normal and hypertrophic fibers. Gastrocnemius of the calf, 7 months of CS_2 exposure. Hematoxylin and eosin 300x.

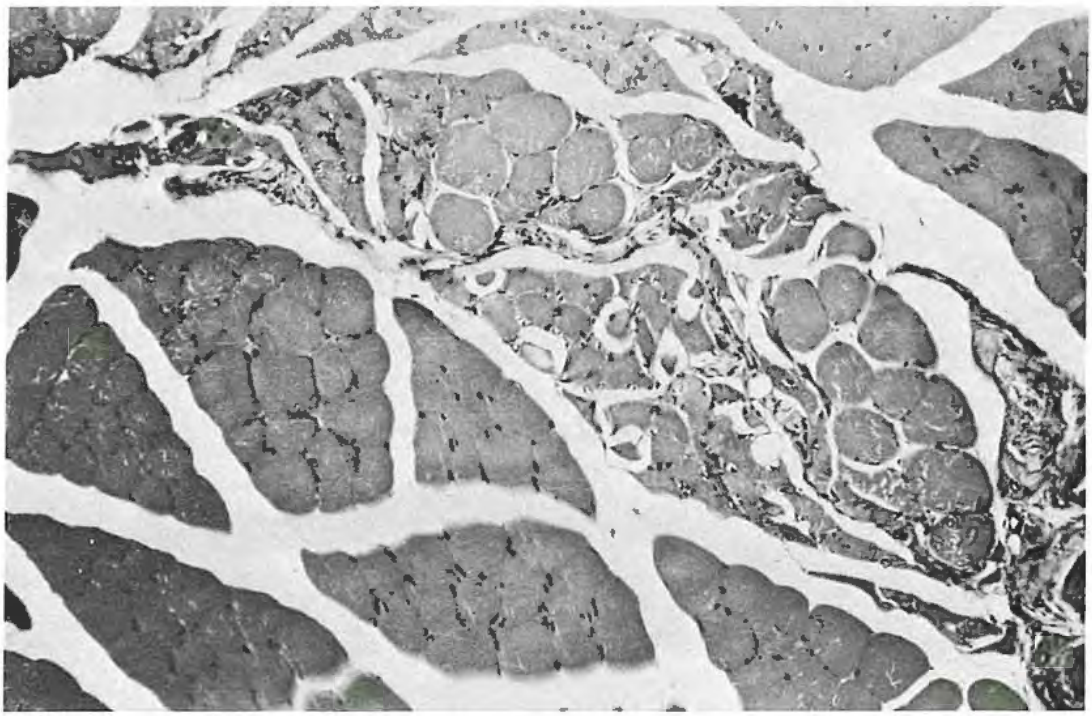


Figure 10. Hypertrophic muscle fibers between the atrophic groups. Gastrocnemius of the calf, 11 months of CS_2 exposure. Hematoxylin and eosin 120x.

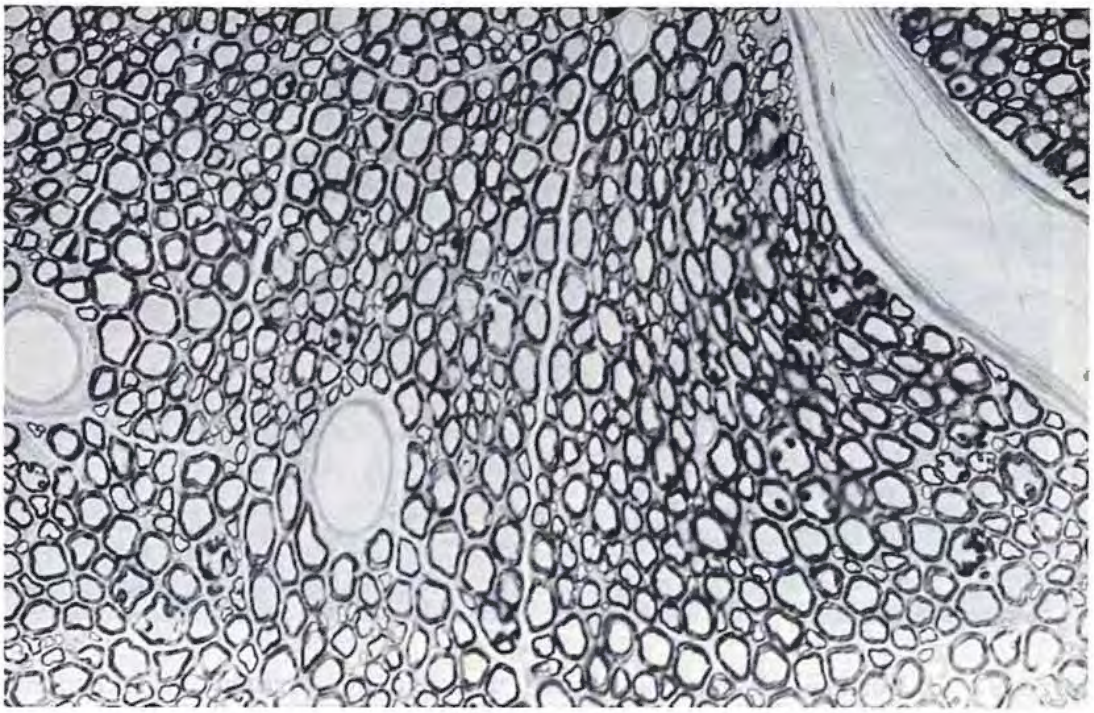


Figure 11. Ischiadic nerve of the control rat, transverse section. OsO_4 impregnation 300x.

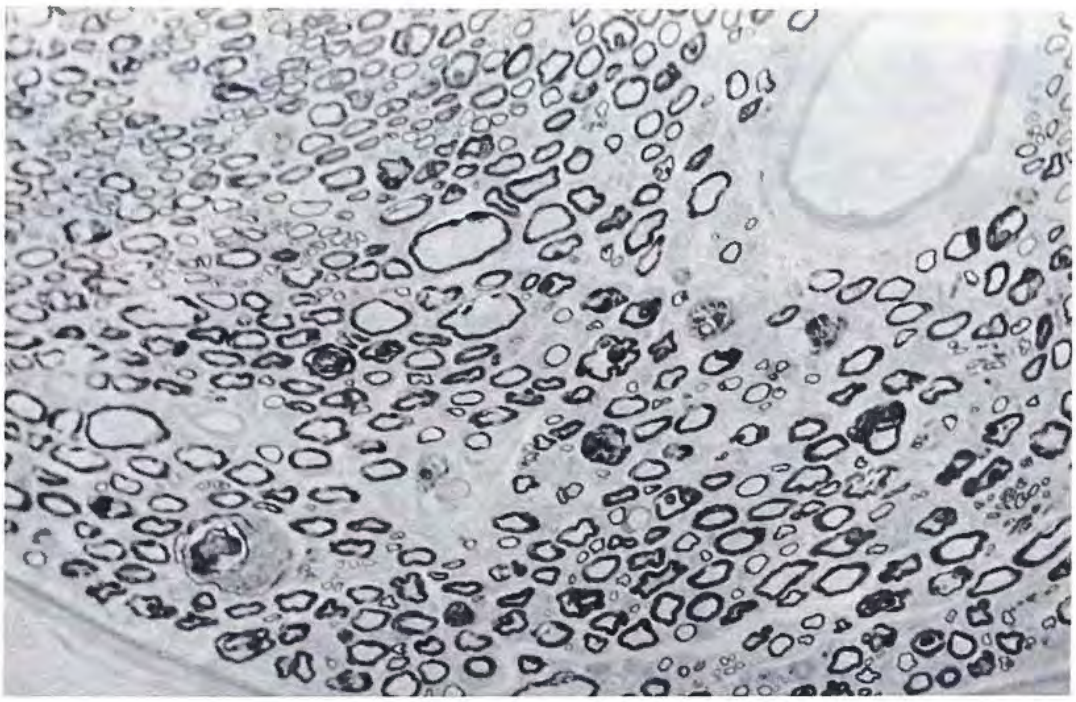


Figure 12. Ischiadic nerve of the rat after 8 months of exposure to CS_2, OsO_4 impregnation.
Note the various diameter of nervous fibers and the amount of intertitium 300x.

PROCEEDINGS

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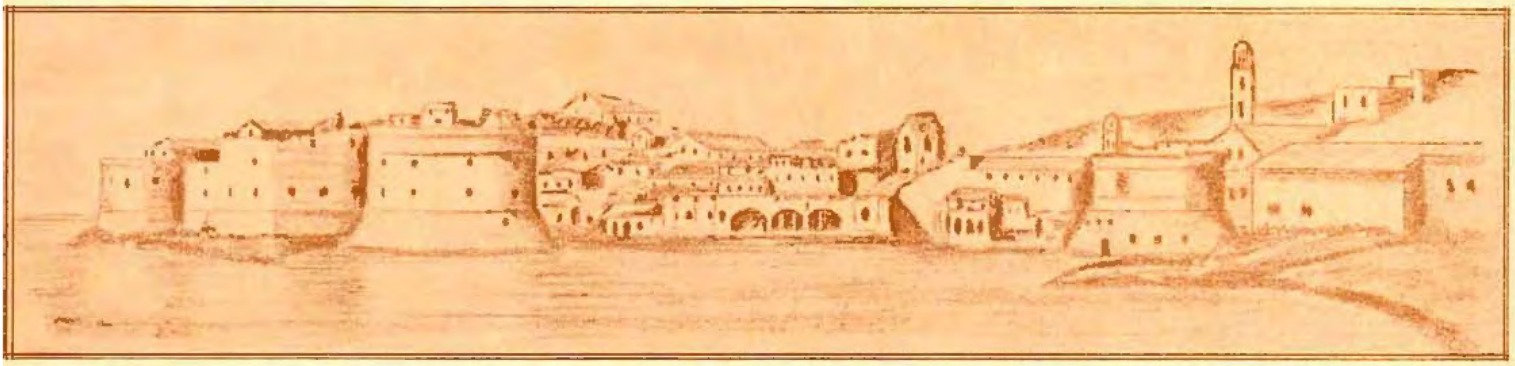

OCTOBER 1970

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