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The Long-Term Effect of Perphenazine Enanthate on the Rat Brain. Some Metabolic and Anatomical Observations

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Abstract. Perphenazine enanthate 3.4 mg/kg per injection, was administered subcutaneously to rats every second week over a period of a year, a total of 31 mg per animal being given. The animals were observed weekly and only a few became cataleptic during brief periods. After treatment for one year, ^{3}H-uridine and ^{3}H-lysine were administered intravenously and the labelling was studied by microautoradiography. Labelling of the cortical cells in the treated animals was found to be slightly greater than in the control animals. The converse was found in the basal ganglia in the case of uridine. None of these differences were significant.

In counting nerve cells in the cortex and in the basal ganglia, a significantly lower number of nerve cells was found in the basal ganglia in the treated group.

Histological investigation of lungs, liver, spleen and kidneys showed only insignificant changes in the tissues.

All the investigations mentioned were performed blind.

Key words: ^{3}H-Uridine — ^{3}H-Lysine — Perphenazine — Microautoradiography — Nerve Cell Counts.

An extensive literature exists on the effect of chlorpromazine on the animal brain following long-term administration (see review by Sommer and Quandt, 1970). A series of changes in the brain have been described, including „degeneration" of nerve cells, satellitosis, neuronophagia, vascular changes and minor haemorrhages (Kemali *et al.*, 1958; Gueyniseman, 1962; Mackiewicz and Gershou, 1964; Popova, 1965). On the other hand, only a few investigators have studied the long-term effect of promazine derivatives on the brain. Romasenko and Jacobson (1969) administered trifluoperazine to rats in doses up to 250 mg/kg, for up to 5 weeks. They found only small morphological changes, which were reversible. In the case of perphenazine, there are a few studies on cerebral changes. Grünthal and Walther-Büel (1960) described pronounced cell changes in the inferior olive in a patient who had suffered from dyskinesia after the administration of perphenazine for 13 days. Nielsen *et al.* (1969) found that this substance produced chromosome changes, while Cohen *et al.* (1972) were unable to confirm these findings. We have administered per-

phenazine to rats over a period of 12 months, and found only small changes in the uptake of ^{3}H-uridine and ^{3}H-lysine in the nerve cells, but a significant loss of cells in the basal ganglia.

Material and Methods

Forty white rats of the Wistar strain were used. The animals were 3 months old at the commencement of the investigation and weighed 240—250 g. Thirty animals were given a subcutaneous injection of perphenatine enanthate, 2.4 mg/kg, every second week for a period of several weeks. As the animals did not become sedated at this dose it was increased to 3.4 mg/kg. The injections were given on the right and left side alternately, and no infiltrates were seen at the sites of injections. The injections were given over a total period of one year and the total amount injected was 30.9 mg per animal. In addition, the oil in which perphenazine is dissolved was injected subcutaneously into 10 animals. These control animals also received injections every second week over a period of one year.

The animals were placed singly in cages, measuring $17 \times 23 \times 28$ cm, made of wire netting, with a mesh size 18×18 mm. The animals received food pellets and a vitamin supplement. Observations of their behaviour in the cages and spontaneous motor activity were made once a week. The animals were weighed every second week; they thrived normally, the weight rising in the course of the year to 450—600 g (mean 490 g). Some of the animals had a brief upper respiratory tract infection, and a few had occasional diarrhoea. Severely affected animals were sacrificed. Towards the end of the experiment, decubitus ulcers on the paws became a problem, presumably due to the coarse wire mesh floor in the cages. There were no infections. A total of 10 animals died or were sacrificed during the experimental period: three animals were sacrificed because of cancer, 2 with pulmonary infection and 1 with persistant diarrhoea. In addition, 4 animals were sacrificed after treatment for 8 months. By the end of the injection period there remained 8 control animals and 22 treated ones.

After the termination of the experimental period, a total of 12 animals were used for the following experiment. Six animals (4 perphenazine animals and 2 control animals) were injected intravenously with 2 mC ^{3}H-5-uridine (specific activity 25 C/mM, Amerham, England) per animal, and the same number of animals were injected with ^{3}H-DL-lysine (specific activity 10.8 C/mM). One hour later, the animals were anaesthetized with chloroform and then sacrificed by perfusion with Lillie's fluid (formalin 4%) via the left cardiac ventricle, the right atrium being clipped open. All the other experimental animals were sacrificed in the same way. All animals were then immersed in Lillie's fluid until next day, after which the brain was removed. This was post-fixed in Lillie's fluid for 2 days, and then each hemisphere was cut into 3 blocks by means of frontal sections, the blocks then being rinsed in water for 24 h, cleared in chloroform, placed in 54° C paraffin overnight, embedded in paraffin and cut into 4 μ sections.

The sections from the 12 tritium-injected animals were de-paraffinized and coated with Ilford Research Emulsion K-5 at 45°. They were dried for 60 min and placed in light-tight boxes with Drierite at 4° C for 4 days in the case of the lysine sections and for 14 days in the case of the uridine sections. The sections were developed with Amidol for 4 min at 18° C and fixed in 30% sodium thiosulphate for 7 min at 18° C. They were then stained with hematoxylin-eosin. Grain counts were made on these autoradiograms over nerve cells in the cortex and in the corpus striatum, the counts being made both over nuclei and cytoplasm and over the neuropil, in the latter in squares of 400 μ^2. The counts were made over

25 cells in the 2nd layer and 25 cells in the 5th layer of the cortex, over 25 cells in the corpus striatum and over 25 neuropil squares in the cortex and corpus striatum. Grain counts were also made over 25 nuclei in the choroid plexus. Counts were made on 2 sections from each animal. The diameters of the nuclei were measured in 3 sections, giving 8.7 μ in the 2nd layer of the cortex, 11.9 μ in the 5th layer of the cortex, and 8.8 μ in the basal ganglia. All grain counts over the nuclei were adjusted for the same nuclear area, so that the counts could be compared directly. All counts were made blind, and were done by the authors (HP and RF). The background activity was determined in 25 squares with an area of 1600 μ^2 per square. As this value was in all cases less than 1% of the count for each area no correction was made for background activity.

Finally, the nerve cells in the cortex and in the basal ganglia were counted in 8 control animals and in 12 perphenazine-treated animals. The counts were made using an ocular micrometer and a 40× objective. Counts were made in squares of 10000 μ^2. In the cortex, counts were made in 4 tracks from the surface down to the white matter. In the basal ganglia, 5 tracks of 10 squares were counted. Counts were made on 2 sections from each animal, and these 2 results were added before making the statistical analysis. All counts were of nucleoli. No correction was made for cutting the object counted, as this is only a question of a comparison between 2 groups of counts. Section thickness was determined by focussing on the upper surface and on the lower surface of the section using the oil-immersion objective, 10 measurements being made on each section and the cell counts being corrected for deviations from the mean tickness (4 μ). All counts were made blind, and were done by the authors (H.P. and R.F.).

The liver, kidneys, lungs and spleen were removed from all 34 animals (30 animals injected for a year, 4 animals injected for 8 months). Per Christoffersen, M.D., Pathological Institute, performed the microscopic examination of these tissues. This evaluation was likewise carried out blind.

Results

Behaviour. The motor activity of the animals was normal during the first few months, with one exception. Later, their movements were somewhat inhibited due to sores on the paws. Periodically they were a little irritable and aggressive. All animals were examined once a week for catalepsy. The animals were placed at the wire netting wall. Normal rats will move up to the edge, but cataleptic rats stay quietly where they are placed. Four animals in the treatment group were more or less cataleptic, one of them from the commencement of the investigation, the others only during the latter half of the experimental period. In 12 animals catalepsy was observed in 58 cases (the total number of observations was 1462). These cases of catalepsy were observed during the whole period of treatment, without any special pattern. Ten of the rats were from the treated group, 2 from the non-treated. A total of 24 animals, 16 treated and 8 non-treated, did not show any catalepsy at any time.

Autoradiography. The results of the grain counts are seen in Table 1. This shows that in the case of uridine, the labelling of nuclei and neuropil in the cortex is slightly greater in the treated animals than in the control

Table 1. Grain counts from animals injected with tritiated uridine or lysine. Each figure is the average of counts in 2 sections. All figures are multiplied by 10

	Cortex							Basal Ganglia			
	small cells			great cells							
	nuclei	cyto-plasm	neuro-pil	nuclei	cyto-plasm	neuro pil	Plexus cells	nuclei	cyto-plasm	neuro-pil	
	140	11	24	47	15	33	48	25	2	20	
	204	14	40	46	9	30	47	26	4	78	
	252	13	29	69	21	28	35	30	4	53	treated
	301	19	41	62	16	41	14	34	3	45	animals
Uridine	224	14	33	56	15	33	36	29	3	49	
	218	13	30	56	23	31	46	33	2	51	
	113	7	23	49	11	22	65	46	7	64	controls
	165	10	27	53	17	26	56	39	4	58	
	56	18	116	65	59	99	23	42	8	80	
	51	11	67	70	72	69	24	40	10	73	
	76	12	87	74	59	84	20	38	9	100	treated
	46	9	59	75	59	60	20	29	8	91	animals
Lysine	57	13	82	71	62	78	22	37	9	86	
	35	6	52	58	43	59	19	32	7	64	
	46	7	65	63	72	68	22	39	7	78	controls
	41	6	59	60	58	64	21	36	7	71	

Table 2. Number of nerve cells per unit area in 12 treated and 8 control animals. Each figure is the average of counts in 2 sections. The difference found in the basal ganglia is significant ($p < 0.005$)

Cortex		Basal ganglia	
treated animals	controls	treated animals	controls
864	1075	825	1112
1019	1057	834	951
1022	828	804	724
955	869	834	866
1018	863	714	818
1111	767	693	997
954	933	626	1012
964	838	780	772
916		659	
1030		725	
833		773	
901		682	
965.6	903.8	745.8	906.5
difference = 61.8	S.E. = 42.3	difference = − 160.7	S.E. = 45.9

animals. In the case of the basal ganglia, the relationship is reversed. None of the differences are significant. The values from the animals injected with lysine are likewise slightly higher in the case of the animals which were treated than in the case of the control animals, this applying to both cortical cells and cells in the basal ganglia, but the differences were not significant. Similar results were obtained for the choroid plexus cells.

Cell Counts. Table 2 shows that the number of nerve cells is significantly lower in the basal ganglia of the perphenazine-treated animals than of the control animals. There is no difference in the number of nerve cells in the cortex in the 2 groups.

Histological Investigation. In the case of the brain, it should be emphasized that there was no difference in the sections from the 2 groups, either with respect to the morphology of the nerve cells or with respect to the placing of the glial cells (satellitosis, neuronophagia). Degenerated nerve cells were not seen, and the cytoarchitectonics in the cortex was normal. The liver was normal, but in 5 cases (2 from the treated group, 3 from the control group), scattered lymphocytes were found in the portal space, a few focal necroses in the parenchyma and slight focal proliferation of Kupffer cells. In animals which have been involved in experiments for a longer period, such changes are not unusual. The changes described show no toxic characteristics. Renal tissue was found to be normal in all cases; no degenerative changes were found, no interstitial fibrosis or inflammatory reaction, and the vessels were normal.

The lung tissue was normal; in most of the cases, rather dense lymphocytic infiltrations were found in the bronchial wall, with a tendency to the formation of reaction centres. This is a normal finding in the bronchial wall of the rat.

The spleen showed larger or smaller groups of macrophages containing haemosiderin. In addition, extramedullary haemopoiesis was found. Both these conditions are normal in the rat.

Discussion

The dose of perphenazine was so adjusted that the animals did not become sedated, and were only slightly cataleptic. Larger doses would have affected the motor behaviour of the animals to such an extent that the reduced mobility over such a long period of time might theoretically have caused changes in the nervous system. Another reason for using relatively small doses was that we wanted to examine the effect of clinically comparable amounts used in schizophrenic patients. It was necessary to use a long acting preparation, as daily injections during a year would have elicited hard „infiltrations" in the skin with absorption problems as a consequence.

Our choice of uridine and lysine was determined by our desire to check some main components of nerve cell metabolism, RNA and protein formation during the perphenazine treatment. The autoradiographic findings may be taken as indicating that both the uridine and the lysine in the cortical cells were taken up in greater amounts in the animals under long-term treatment than in the control animals. It has been shown previously (Pakkenberg and Fog, 1972) that uridine-labelling is at a maximum 30 min after intravenous administration, and that there is no fall in the labelling until 12 hours have elapsed after administration. As the animals in the perphenazine investigation were sacrificed one hour after administration, it is impossible on the basis of the present data to know whether there are also changes in the rate of uptake and in the half-life.It should be mentioned that the uridine labelling in the basal ganglia of the treated animals is less than in the control animals. The differences are small, but as they are in the opposite direction to the differences in the cortex, they may nevertheless be significant. There may be a question here of either a slower uptake or an increased metabolism — or of both, and only kinetic studies can decide this question. Rösner (1972), studying the optic tectum in teleosts, has found a reduced labelling of nerve cells following the administration of uridine and histidine in animals treated with chlorpromazine. Kinetic studies will be necessary in many brain regions in order to achieve a real overall view of this question.

It might be mentioned here that the far greater number of grains per unit area of the nucleus in the small cells of the 2nd layer of the cortex in uridine-injected animals, compared with the values found for the large nerve cells in the 5th layer, is a new observation which requires further investigation.

The most significant result in the present investigation is the decrease which has been demonstrated in the number of nerve cells in the basal ganglia in animals under long-term treatment. The possibility was present that brain oedema in the treated group might give rise to an apparent decrease in the number of cells per unit volume. With the histological technique employed, however, using perfusion of the brain, brain oedema can easily be recognized, but was not found. The reduced cell number appears to be a real observation — in other words, expressing a loss of nerve cells during the experimental period. It is remarkable that no increase is found in the number of glial cells, or of degenerating nerve cells. However, the number of glial cells is only estimated, and is therefore very uncertain. It is possible to explain the absence of obviously damaged cells by assuming that the loss of cells has taken place only at the commencement of the treatment.

It should be recalled that in schizophrenia brain atrophy is often found. It is doubtful, however, whether treatment with phenthiazine derivatives alone can be the cause of this, as cerebral atrophy was also found in schizophrenia before treatment with these drugs was introduced.

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