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THE ANATOMICAL EFFECT OF CERTAIN DRUGS ON THE PURKINJE
CELLS OF THE CEREBELLUM.

by

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THE EFFECT OF CERTAIN DRUGS ON THE PURKINJE CELLS
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OF THE CEREBELLUM

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The purpose of this series of experiments is to determine if certain drugs produce structural changes in the higher brain cells, of which the Purkinje cell is taken as a type. It is known from physiological experiments and the ordinary experience of life that certain drugs do produce functional activity, but whether these functional changes are accompanied by anatomical changes in the brain cells, is unknown. If there are any anatomical changes, are these the changes of normal functional activity? The two drugs used in this series of experiments were alcohol and caffeine. The reason for choosing these drugs was because of their importance, due to their wide use. Alcohol is contained in many medicines and beverages, while caffeine is found in tea and coffee, and at present in many summer drinks that are dispensed at the soda fountains.

EXPERIMENTAL METHODS. The rabbit was the animal chosen

for this work, largely on account of the fact that it is easily obtained and easily handled. Young adult rabbits were used in every case. Several methods were tried for giving the animals alcohol but the method found to be the most satisfactory was by means of the stomach tube. A stick was placed in the animal's mouth, a soft rubber catheter passed into the ~~animal's~~ stomach, and the required amount of alcohol poured in. A ten per cent ^{solution} was used in all experiments, unless specified otherwise. After the first pas-

sage of the catheter the animals seemed little excited by any subsequent passages. Since this was the case the possibility of excitement changes may be disregarded. Nineteen animals were used in these experiments. Results suitable for tabulation were obtained in nine cases. The others were unfit for tabulation due to the fact that the animals died during the night or at some other time when the operator was absent from the laboratory, and the brain, because of post-mortem changes, was not used in the tables given in this article. However sections were taken from these brains and studied. The post-mortem changes were easily distinguished, and making allowances for these, the changes seen in the Purkinje cells were the same as obtained in the other animals. The experiments upon which the tables of this article are based, may be divided into three general classes, according to the amount of alcohol given and the duration of the experiment. These are normal, acute, and chronic. There are three experiments of each class. With one exception the animals were killed by a blow on the back of the neck. Rabbits numbered 0, 6, and 9 are normals hence were taken from the pen and killed without treatment of any kind. The data for these experiments is set forth most conveniently in table form, showing, date, time, and dosage.

EXPT. No. 1. Acute alcohol. White rabbit. Weight 1480 grams.

Duration of experiment 24 hours. Total amount of alcohol given, 150 cc. of 20%.

In giving the second dose of alcohol the animal drew some of the fluid into its lungs and died. The brain was at once removed.

EXPT.No.7.Acute alcohol.White rabbit.Weight 1650 grams.Duration of experiment 48 hours.Total amount of alcohol given 360 cc.Average per day 180 cc.

Mar.19,'12	9a.m.	75 cc.	Mar.20,'12	3a.m.	30 cc.
" " "	noon	50 cc.	" " "	9a.m.	30 cc.
" " "	4p.m.	50 cc.	" " "	2p.m.	100 cc.
" " "	100p.m.	75 cc.	Killed at 4 p.m.		

EXPT.No.11.Acute alcohol.White rabbit.Weight 2200 grams.Duration of experiment 56 hours.Total amount of alcohol given 725 cc.Average per day 300 cc.

Mar.18,'12	10a.m.	50 cc.	Mar.19,'12	4p.m.	75 cc.
" " "	2p.m.	75 cc.	" " "	10p.m.	75 cc.
" " "	8p.m.	75 cc.	" 20 "	8a.m.	50 cc.
" 19 "	9a.m.	75 cc.	" " "	2p.m.	100 cc.
" " "	noon	50 cc.	" " "	4p.m.	100 cc.

EXPT.No.8.Chronic alcohol.White rabbit.Weight 2140 grams.Duration of experiment 7 days.Total amount of alcohol given 860 cc.Average per day per 1000 grams weight 58 cc.

Feb.9,'12	9a.m.	40 cc.	Feb.13,'12	2p.m.	70 cc.
" 10 "	10a.m.	70 cc.	" 14 "	10a.m.	80 cc.
" 11 "	10a.m.	70 cc.	" 14 "	5p.m.	50 cc.
" 12 "	10a.m.	50 cc.	" 15 "	9a.m.	90 cc.
" 12 "	2 p.m.	70 cc.	" 15 "	3p.m.	60 cc.
" 13 "	10a.m.	60 cc.			

EXPT.No.10.Chronic alcohol.White rabbit.Weight 2170 grams.
Duration of experiment 18 days.Total amount of alcohol
given 1920 cc.Average dose per day per 1000 grams wt.49 cc.

Feb.28.'12	9a.m.	80cc.	Mar.4,'12	2p.m.	40 cc.
" " "	4p.m.	70cc.	" 5 "	9a.m.	60 cc.
" 29 "	9a.m.	60cc.	" 5 "	3p.m.	40 cc.
" " "	3p.m.	60cc.	" 6 "	9a.m.	80 cc.
Mar.1.'12	9a.m.	60cc.	" 6 "	3p.m.	40 cc.
" 2 "	10a.m.	60cc.	" 7 "	10a.m.	100 cc.
" 3 "	4p.m.	70cc.	" 7 "	3p.m.	40 cc.
" 4 "	10a.m.	80cc.	" 8 "	10a.m.	100 cc.
" 9 "	2p.m.	100cc.	" 13 "	10a.m.	100 cc.
" 10 "	2p.m.	70cc.	" 14 "	10a.m.	100 cc.
" 11 "	10a.m.	100cc.	" 15 "	10a.m.	160 cc.
" 12 "	10a.m.	100cc.	" 16 "	noon	160 cc.

The animal was killed at 3 p.m.

EXPT.No.5.Chronic alcohol.Gray white rabbit.Weight 1610
grams.Duration of experiment 14 days.Total amount of alcohol
given 1550 cc.Average dose per day per 1000 Gms.wt.68cc.

Mar.19,'12	9a.m.	75cc.	Mar.26,'12	4p.m.	80cc.
" " "	4p.m.	75cc.	" 27 "	10a.m.	80cc.
" 20 "	10a.m.	75cc.	" 27 "	4p.m.	80cc.
" 21 "	10a.m.	75cc.	" 28 "	10a.m.	100cc.
" 22 "	10a.m.	75cc.	" 29 "	10a.m.	80cc.
" 22 "	10a.m.	50cc.	" 29 "	3p.m.	40cc.
" 23 "	11a.m.	75cc.	" 30 "	3p.m.	100cc.
" 24 "	11a.m.	100cc.	" 31 "	10a.m.	80cc.
" 25 "	10a.m.	80cc.	Apr.1.'12	9a.m.	100cc.
" 25 "	4p.m.	50cc.	" 1 "	2p.m.	100cc.
" 26 "	10a.m.	80cc.			

As soon as the animal was killed, the brain was removed and sections taken from the uvula, worm, biventral and posterior inferior lobes of the cerebellum and from the medulla, spinal cord, basal ganglia, spinal ganglia, and Gasserian ganglia. The fixing fluid used was a solution composed of 10cc. of 40% formalin to 90cc. of saturated bichloride of mercury solution. The tissue was left in this solution from three to five hours, and was then run thru the graded alcohols to absolute alcohols and xylol. IN the 50, 70, and 80% solutions, iodine was added to remove the mercury. The tissue was then imbedded in paraffin and sections, five micra in thickness, cut and mounted by the water method. The paraffine was removed in the usual way and the sections stained from one to two minutes in a one per cent solution of erythrosin; then washed in water and stained five to seven minutes in a saturated solution of toluidin blue. The stain was differentiated in 95% alcohol, and then passed thru absolute alcohol and xylol and mounted in Canada balsam.

In order to interpret the changes, if any, that are produced by alcohol, it is first necessary to consider the normal animals and the changes of normal activity. Considerable work has been done by different writers on functional activity and all have found decided changes. Within reasonable limits their findings have been the same but their interpretations vary widely as to the meaning of these changes. These writers will be cited in connection with the part

of the nervous system studied. Sympathetic ganglia only, Eve ('96), Lambert ('93), Lugaro ('95), Vas ('92); Cord only, Holmgren ('00), Pick ('98); retina only, Chizzini ('06), Spinalganglia and cortex (latter not in detail), Hodge ('92-'94); Cortex without regard to other parts, Guerrini ('99-02), Mann ('95), Nissl ('94-'96) and Van Durme ('01). Berkley ('95), Andrieszen and Dehio.

Notwithstanding the differences of these writers at least two of them confirm every important process of cell change. All agree that there is a change in chromatin content, and also that there is size changes. Hyperchromatism is regarded as an indication of initial activity by Holmgren, Lugaro, Odier, and Vas. Of the others, some explicitly, as Mann, Nissl ('06) and Van Durme, refer, to this state as one of repose, while others seem to think that it is a starting point for activity, if not activity itself. Nissl ('94) earlier regarded the pyknomorphic smaller cell as indicating fatigue. Hyperchromatism, chromatolysis, is universally considered to accompany, in progressive degree, activity and fatigue. Lugaro and Vas determined an initial augmentation in size in association with hyperchromatism. Guerrini (nucleus), Holmgren, Mann (nucleus for cortical cells only) and Van Durme agree on an enlargement during chromatolysis. This point caused great misunderstanding, due to the fact that most of these writers based their opinion on a few stages of the cell changes. If the whole cycle be traced it will be seen that there are two distinct periods of enlargement, one at the beginning, the other in

the later stages, the one hyperchromatic, the other hypochromatic. Thus it will be seen that while some writers found an increase in size during the hyperchromatic period and based their conclusions on this fact, others found the increase in the hypochromatic cells and based their conclusions on this phase of the cycle. No one of these writers went far enough in the cycle of changes to see both and base his conclusions on this foundation.

All of these changes have been confirmed and given a definite place in the process of activity by Dolley. He divided the cell cycle into thirteen stages according to size and chromatin content, as follows:

(1) The resting cell. It is lacking in intranuclear chromatin except within the karyosome (nucleolus) and the amount of extra nuclear chromatin varies with the individual.

(2) The stage of progressive hyperchromatism, in which in the pure type, the initial enlargement of the whole cell reaches its maximum.

(3) The stage of maximum hyperchromatism which is associated with the beginning of shrinkage.

(4&5) The stages of regressive hyperchromatism together with the maximum of shrinkage. Both stages four and five can be further divided into an early or pure Hodge type and a late division, characterized by the sharp beginning of nuclear oedema.

(6) The return of cytoplasmic chromatin in its continued

reduction to the average normal level. This stage is principally distinguished, morphologically by the maximum disproportion in size of the nucleus owing to its much greater oedema.

(7&8) The stages leading to the primary disappearance of cytoplasmic chromatin.

(9&10) The stages of secondary restoration of cytoplasmic chromatin. The chromatin is first piled up around the nuclear membrane and then passed out.

(11) The stage of secondary disappearance of cytoplasmic chromatin, with the complete using up of the previous supply. The karyosome is left containing only a vestige of basic chromatin and the cell is much more exhausted looking.

(12) The disintegration and passing out of the ultimate content contained within the karyosome.

(13) The exhausted cell.

The first step made by the writer was the confirmation of the existence of these chromatin and size changes. This was done by examination and a series of cell measurements. These measurements were made in the following manner: ten cells typical for each stage were sketched by means of a camera lucida and diameter measurements made in millimeters. From these figures the comparative cell and nuclear volumes were calculated, an average being struck for the ten cells of each stage. Following the method used by Popoff and Dolley the volumes were calculated in

in terms of the corresponding parallelopipeds, the third dimension being taken as the shorter diameter. To obtain the volume of the cell protoplasm the nuclear volume was subtracted from the total volume. The plasma volume divided by the nuclear volume gives the coefficient of the nucleus-plasma relation. From these figures curves were constructed both for the volume of the nucleus and cell and also one for the nucleus plasma relation. The curves obtained are shown in Figure one and for comparison, the curves constructed by Dr. Dolley ^{for the dog} are also shown (Figure 2). These curves will be seen to be almost identical, which is the more remarkable from the fact that Dr. Dolley's curves were constructed by averaging from twenty five to fifty cells, while the writer's curve was obtained from averaging ten cells to the stage. This shows that the volumes of the cell and nucleus must run very nearly constant for the various stages.

From these measurements (Fig. 1) it will be seen that there are two distinct periods of enlargement, the first in stage two, the other in the latter stages; the one hyperchromatic, the other hypochromatic. The course of the size changes of both cell body and nucleus may here be conveniently summarized together. The transition from the resting stage to activity is characterized by a marked increase in size, which reaches a maximum in the pure type of stage two. In its size relations, the remarkable fact is that the cell body and nucleus increase in the same proportion, that is, the nucleus-plasma relation remains the same.

as in stage one. From this point both the cell body and nucleus decrease in size until stage five (Hodge's type) is reached, but the decrease of the nucleus and cell body is not in proportion. The nucleus progressively becomes smaller and smaller in relation to the cell body. From this point it is sufficient to say that the nucleus and cell body increase in absolute size. In stage six however the nucleus has increased much more, in proportion than the cell body, hence the nucleus-plasma relation is low, in fact, the lowest of any stage of the cycle. From this point the increase in the nucleus is relatively less than the cell body, hence the nucleus plasma relation rises. This increase in size continues until the cell is finally exhausted. The karyosome disappears and the outline of the cell and nucleus becomes indistinct.

In making conclusions in this ^{5th} experiments the cells of the alcohol animals were first gone over to determine the extent, if any, of the chromatin and size changes produced. Both the chromatin and size changes were identical with those of the normal animal, stage for stage. To arrive at a conclusion a count of six hundred diagnosed cells was made on each animal and also a record kept of the undiagnosed cells as to whether they were hyperchromatic or hypochromatic. Since there are slight variations in individual animals, averages were struck for each of the three different classes, that is, one for the acute, one for the chronic and one for the normals. (See table A).

From this table it will be seen that the normal and acute animals run very nearly the same. Classing the stages one to five as distinctly hyperchromatic, it will be seen that the acute alcohol animal totals only ten more of this class than the normal. Then classing cells nine to thirteen as distinctly hypochromatic it is seen that the acute alcohol animal has nineteen more of this class. In the undiagnosed cells it will be seen that the ratio in the acute alcohol animal is 611 hyperchromatic to 376 hypochromatic, while in the normal animal the ratio is 452 hyperchromatic to 397 hypochromatic. In connection with this comparison it would be well to say that one of the acute alcohol animals received much less alcohol than the other two. Of the hypochromatic cells two of the animals showed 192 and 179 respectively, while the other showed only 138. This would seem to indicate a higher state of activity in the case of two of the animals ^{compared} both with the third and with the normals. Upon comparing the normals with the chronic alcohol animals a striking change is at once seen. The number of cells in the hypochromatic group is seen to be very much greater in the chronic alcohol animals than in the normals, the average ratio being 356 in the alcohol animal to 150 in the normal animal. For the hyperchromatic stages the reverse is true the normal animal having by far the greater number of this class, the ratio being (306 to 128). By these figures it will be seen that the cells of the chronic alcohol animals are in

a strikingly more advanced stage of activity than the normal animal.

Taken as a whole little work has been done on the effect of alcohol on the brain. Probably the most extensive work has been done by Berkley, whose article appears in the "Journal of Neurology", 1895. In his work he did not classify the cells in a definite cycle but merely compared the appearance of the cells of the alcohol brain to those of the normal. His animals were all chronic cases and while the duration of the experiment was longer the amount of alcohol given per day was considerably less. He used the osmic acid stain. "The Purkinje cells stained well, often an entire row being blackened and exquisitely brought out. When an entire row was taken the proportion was found to be three normal to one degenerated cell." "The motor cells are swollen, rounded, stain deeply, become granularly pigmented, and the apical processes degenerate. The cellular wall is thickened. There is a considerable quantity of pigment deposited between the shrinking protoplasm and the cell wall. The processes of the cells are stunted and covered with nuclei and the cell protoplasm is granular and vacuolated." "The degeneration of the Purkinje cell, while having the same characteristics as the cell degeneration in the degeneration of the cerebrum, is much more striking, and there is no possibility of mistaking the process for anything else than it actually is, - a degeneration of cellular structure." Thus it will be seen that allowing for differences in stain, the anatomical changes produced appear almost identical with those obtained by the writer

but our interpretations of them is somewhat different. Certain of his changes, such as the pigment, are found as well in the normal ^{senile} cell ~~as in those in the state of fatigue.~~

The changes he describes look very much like degeneration but in view of the cycle established on the normal animal, in spite of Berkley's denial of mistaking the process for anything else than a degeneration, I believe that the process is one of over-stimulation rather than a degeneration, as the word is popularly used.

Andrieszen worked on chronic alcoholism in the human brain and obtained the following results. He stained with toluidin, Nissl and Golgi. Not all of the cells in the alcoholic brain show equally advanced changes. While some show moderately advanced intercellular changes, at once recognizable as such, others, upon whom, for some reason, stress has not fallen so severely, will show appearances consistent with health or only faint indications of a commencing departure from the normal. He concludes, (1) that there is an increased functional activity in the cell body, (2) this is productive of a degeneration or degradation in the form of nerve energy. But his interpretation of hyperchromatism is that it means exhaustion and degeneration while we believe it to be the beginning of cell activity. Their interpretations of these changes is degeneration, in other words pathological, while my interpretation puts them on a physiological basis of natural effects produced by natural stimulation. This makes the end result none the less degen-

erative, but it gives a more logical explanation of the effects of alcohol.

Vas injected dogs daily with alcohol for six to twelve weeks. The result was a central chromatolysis. He regarded these changes to be the result of general malnutrition rather than specific for alcohol.

Dehio administered fatal doses of alcohol thru the stomach but no definite alterations were observed in the brain cells. When the animal lived from eighteen to thirty six hours, characteristic changes were found effecting the whole or part of the body of the cell. The chromatic network of Purkinje's cells was replaced by many fine granules irregularly arranged, while the achromatic substance stained diffusely light blue. Many normal cells were found.

Since we do find the changes discussed in this paper it would be well to advance a theory in accordance with our work as to their cause.

(1) ^{more likely is} The theory that these changes are brought about by the actual presence of alcohol in the blood. This theory seems plausible. It is a well known fact that the kidneys, liver, and other organs are effected by the use of alcohol. In the case of the kidneys at least this appears as the nature of a stimulation. Since these organs do not come in contact with the alcohol except thru the blood, it is reasonable to suppose that alcohol might have a similar effect on the brain cells. In the kidneys the result, anatomically, is a cloudy swelling. Adami and other pathologists have agreed

that this condition is caused by the over stimulation of a cell. In chronic alcoholism the nerve cells are in a state of marked exhaustion, which presupposes a stimulating effect, and the analogy with the kidney bears this out.

(2) The other theory is that the effects are produced by toxins absorbed from the alimentary tract, ~~the~~ alcohol having impaired its functioning power. In all cases however where the functioning power of the alimentary tract is disturbed, corresponding results are not found in the brain cells. The exact actions of toxins is not known and in the present state of ignorance, it is impossible to draw any definite conclusions along this line.

The conclusions drawn are three:

(1) That anatomical changes are produced in the Purkinje cells by the chronic use of alcohol and that these changes are those of normal functional activity.

(2) In acute alcoholism if any structural changes are produced they are those of a slight stimulation of the cells.

(3) That in chronic alcoholism the cells show a high degree of over stimulation and over activity as compared with the normal animal.

Section

Rabbit	Normal			Acute			Chronic			Average		
	0	6	9	1	11	7	8	10	5	NORMAL	ACUTE	CHRONIC
Stage 1	31	7	30	30	7	2	12	1	2	23	13	5
2	12	6	11	47	3	4	2	8	5	9	18	5
3	62	120	120	102	111	68	40	19	33	101	94	31
4	177	176	168	129	199	242	68	95	98	173	190	87
5	53	29	68	37	26	50	22	32	62	50	38	37
6	50	47	21	60	47	39	27	27	52	37	49	35
7	37	30	17	25	6	7	22	26	6	28	13	18
8	40	19	10	32	10	9	4	31	17	23	17	14
9	52	35	27	31	36	23	39	115	26	38	30	60
10	55	57	68	67	78	92	86	135	94	60	79	105
11	24	56	40	32	66	49	50	90	116	40	49	85
12	1	12	15	8	12	14	26	11	73	9	11	37
13	0	4	5	0	0	1	202	10	16	3	0	79
Hyper	293	239	354	986	445	412	93	127	158	462	611	126
Little	330	298	186	432	309	378	146	505	368	307	376	337

TABLE "A"
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Caption?

Figure No. 2 (Dolby)

Figure No. 1

Lacks adequate explanation.

Caffeine.

The experiments on caffeine were not as numerous as those of alcohol but the results were more striking. The animals were given the drug in a five per cent solution in the same manner the alcohol was administered. (See next page)

The physiological effects of caffeine are well known. When given in small amounts there is a feeling of elation and an increased capacity for work. When given in toxic doses it is followed by tremors, and if in very large doses, by convulsions and death. It is also a diuretic. When given to a rabbit in small amounts, there was little noticeable effect except on the rate of the heart and respiration. In very large doses it caused the typical tremors and in sufficiently large doses, convulsions and death.

Upon examination of the sections from the brain of the animal, which was given small doses for fifteen days, the cells showed an identity stage for stage with the normal but the extent of the changes was much greater. Upon comparison, the caffeine animal showed hyperchromatic cells in ratio of 291 to 306 as compared with the normal; in the hypochromatic stages a ratio of 244 to 150. Thus it may be seen that the cells of the caffeine animal show a much more exhausted condition than the normal.

The results obtained in the case of the animal that was given toxic doses and finally died in convulsions from the effects of the drug, are very much different. In this animal the size changes in the cells were the same as in the

EXPT. No. 12. Chronic caffeine. Weight 2150 grams. Duration of experiment fifteen days. Total amount of caffeine given 4300 mgs. Average per day per 1000 grams weight, 132 mgs.

March 19 '12	10 am.	75 mg.	March 27 '12	10 am.	200 mg.
" 19 "	4 pm.	75 mg.	" 27 "	4 pm.	200 "
" 20 "	10 am.	75 mg.	" 28 "	10 am.	200 "
" 20 "	4 pm.	75 mg.	" 28 "	3 pm.	200 "
" 21 "	9 am.	75 mg.	" 29 "	10 am.	200 "
" 21 "	3 pm.	75 mg.	" 29 "	3 pm.	200 "
" 22 "	10 am.	75 mg.	" 30 "	10 am.	200 "
" 22 "	3 pm.	75 mg.	" 30 "	3 pm.	200 "
" 23 "	noon	100 mg.	" 30 "	3 pm.	200 "
" 24 "	11 am.	100 mg.	April 1 "	10 am.	200 "
" 25 "	10 am.	100 mg.	" 1 "	3 pm.	200 "
" 25 "	4 pm.	100 mg.	" 2 "	10 am.	200 "
" 26 "	10 am.	100 mg.	" 2 "	4 pm.	200 "
" 26 "	4 pm.	100 mg.	" 3 "	10 am.	200 "

Animal killed by blow on the back of neck.

EXPT No. 4. Acute caffeine. Weight 2250 grams. Duration of experiment seven days. Total amount of caffeine given 9100 mgs. Average per day per 1000 grams weight, 577 mg.

March 26 '12	10 am.	400 mg.	March 29 '12	10 am.	1000mg
" 26 "	4 pm.	400 "	" 30 "	10 am.	1000mg
" 27 "	10 am.	600 "	" 31 "	10 am.	1000mg
" 27 "	4 pm.	500 "	April 1 "	10 am.	1000mg
" 28 "	10 am.	1000 "	" 1 "	2pm.	2000mg

The animal died in convulsions at four pm.

normal animal but the changes in the chromatin were entirely different. The cells stained a reddish purple. In a large number, the karyosome stained poorly and was broken into fragments. In many of the cells there was ~~no~~ visible extra ^{-nuclear} chromatin, while in others it appeared as reddish purple splotches rather than clear cut particles with the typical blue stain.

Judging from the size changes and considering the other caffeine animal as a control, the primary effect of the caffeine, even in a toxic dose, must be in the nature of a stimulation. The changes in the cells in this animal seem to be exactly the same as those found in the heat exhaustion experiments performed by Mr. F. A. Martin and presented in his thesis for 1912. In this case the changes ~~do~~ have not been carried to such a marked degree. He finds the same subnormal amount of extra nuclear chromatin and disintegration of inter-nuclear chromatin. In addition he notes ^{no} ~~a~~ diminution of nucleolar substance. This is apparently true for caffeine as well but is not as definite as in heat exhaustion, on account of the less striking degree of change. In heat exhaustion it was interpreted as anatomically an indication of depression resulting from the physiologically poisonous action of waste products. From the two cases of caffeine poisoning there is indication that a similar effect is produced. It goes beyond a stimulation and ultimately disturbs the functional coordination between cell and nucleus. From what is known of the

more specific action of càffêine on nervous tissue this would seem to be the direct effect of caffeine rather than an indirect effect from the production of waste products from other organs. The data is rather me^ag[^]es and this point demands further work.

Conclusions.

- (1) In caffeine, when given in moderate doses, the changes resulting are those of normal functional activity.
- (2) In small doses, long continued, it causes a marked Exhaustion of the Purkinje cells.
- (3) In toxic doses it causes, (a) a stimulation of the cell, (b) destroys the coordination between cell and nucleus, that is, causes depression.

Table "A" ,showing the comparison of counts on normal and chronic caffeine animals. In the case of the acute animal, the cells could not be diagnosed accurately so a count was not made.

Rabbit No.		NORMAL	12.
Stage	1	23	5
"	2	9	2
"	3	101	115
"	4	173	169
"	5	50	35
"	6	37	8
"	7	28	5
"	8	23	18
"	9	38	64
"	10	60	90
"	11	40	78
"	12	9	9
"	13	3	3
Hyper		462	479
Little		307	304

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