AN ANALYSIS OF DNA LOSS AND SYNTHESIS IN THE RAT ADRENAL MEDULLA NUCLEI UPON COLD STIMULATION

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ABSTRACT

The peculiar changes previously observed in DNA content of rat adrenal medulla cell nuclei upon intermittent cold exposure (15 hr at +4°C followed by 9 hr at room temperature) have been further studied with the aid of Feulgen histophotometry and H_3-thymidine radioautography. The amount of DNA decreases progressively with increasing length of cold exposure until 300 hr (−32%). Later a rapid change takes place, whereby DNA content per nucleus returns to values which are slightly, but consistently lower than normal. At termination of a period of cumulative exposure to cold, an analysis of a whole-day experimental cycle shows that the DNA decrease is due to loss of DNA during cold exposure and that DNA synthesis occurs upon return to room temperature. The balance between these two processes can be divided into three stages: (*a*) loss of DNA up to 300 hr of cumulative cold exposure; (*b*) marked increase in DNA by 350 hr; (*c*) oscillation around zero or slightly negative at 400 hr and beyond. These variations are due to: (1) the extension of DNA synthesis into the period of cold exposure as clearly demonstrated by radioautography (stage *b*), and (2) a later still greater DNA loss (stage *c*) which partly offsets the increased synthesis. A complex pattern of adaptation of the adrenal medulla cells, as regards DNA content, to the repetitive cold stimulus is thus demonstrated.

INTRODUCTION

In a previous investigation (1, 2), it has been shown that adrenal medulla nuclei of rats intermittently exposed to cold (15 hr a day) undergo a marked decrease of their DNA content. The decrease is already apparent after 100 hr of cumulative exposure to cold (25%) and reaches 40% after 300 hr. If the animals, after cold exposure, are kept at room temperature, the DNA content per nucleus comes back to normal and supranormal values.

These findings were strengthened by a radioautographic study with H_3-thymidine (3, 4). No significant labeling of adrenal medulla nuclei is detectable in normal rats or in those given H_3-thymidine during cold exposure (at least within 300 hr of the latter), whereas labeling of the nuclei takes place if the radioactive precursor is given to rats during the recovery period (at 18–20°C) after 300 hr of cumulative cold exposure.

These data must be considered as unequivocal evidence that DNA synthesis is taking place during the recovery period. The rate of DNA synthesis is fastest during the first hours of the recovery period, slows down thereafter, and is no longer detectable if the animals are given H_3-thymidine after 5 or more days of recovery.

Taking into account the histochemical observations of Cramer (5) on the marked increase in secretory activity of the adrenal medulla cells of mice suddenly exposed to cold, our data can be

TABLE I

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 50 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	50.14 ± 0.82	50.20 ± 0.83	+0.12	47.88 ± 0.73	−4.62
	b	47.98 ± 1.02	49.84 ± 0.70	+3.88	45.42 ± 0.73	−8.87
	c	48.50 ± 1.11	49.58 ± 0.72	+2.23	45.40 ± 0.73	−8.43
2	*a*	49.22 ± 0.74	49.96 ± 0.67	+1.50	45.02 ± 0.75	−8.99
	b	49.16 ± 0.65	49.66 ± 0.62	+1.02	48.14 ± 0.65	−3.06
	c	48.84 ± 0.54	49.40 ± 0.54	+1.15	45.40 ± 0.58	−8.10
3	*a*	47.22 ± 0.72	50.00 ± 0.60	+5.89	45.84 ± 0.56	−8.32
	b	49.10 ± 0.63	50.38 ± 0.82	+2.61	48.70 ± 0.25	−3.34
	c	49.26 ± 0.49	53.72 ± 0.87	+9.05	43.66 ± 0.71	−18.73

a, Values at the end of 50-hr exposure to cold.

b, Values after additional 9-hr stay at room temperature.

c, Values after additional 9-hr stay at room temperature plus 15 hr at 4°C.

N = normal; E = experimental; K = kidney.

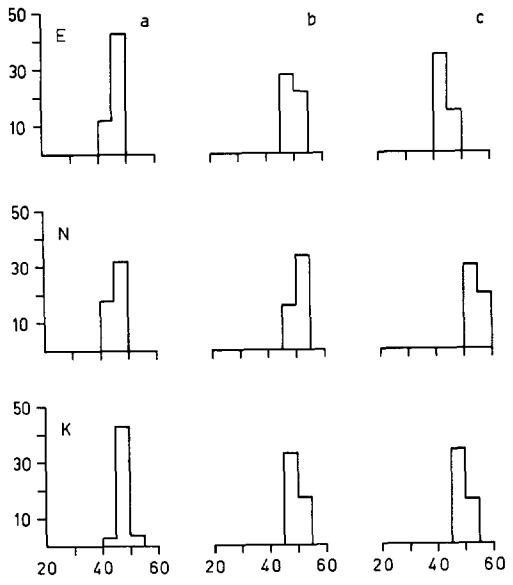

FIGURE 1 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 50 hr of exposure to cold (subgroup 3). *K*, kidney, *N*, normal adrenal medulla, *E*, experimental adrenal medulla. *a*, Values at the end of 50 hr of exposure to cold. *b*, Values after an additional 9-hr stay at room temperature. *c*, Values after an additional 9-hr stay at room temperature plus 15 hr at +4°C.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

interpreted as showing that the reversible changes in the DNA content of the nuclei are related to the functional activity of the cells. This settles in the affirmative, at least in the case of the adrenal medulla, the long-debated question of whether, in fact, such variations occur (6–8, 2).

The availability of an experimental procedure by which gradual variations in the amount of DNA per nucleus can be induced prompted further study of the phenomenon, in order to acquire better information about the relationship between the intermittently applied cold stimulus and the DNA changes, and about the mechanisms involved in the latter.

In the present study the DNA changes have been investigated with histophotometric and radioautographic techniques on short time intervals (respectively, 15 hr of cold exposure and the following 9 hr of recovery at room temperature) after various periods of cumulative exposure to cold (ranging from 50 to 750 hr). It appears that the maximum decrease of DNA per nucleus occurs after 300 hr of cumulative exposure to cold, and that beyond 350 hr the decrease is considerably reduced. The DNA changes observed are due to a shifting balance between DNA loss and DNA synthesis. This shows that a process of adaptation to the experimental stimulus occurs in the adrenal

TABLE II

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 100 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	43.36 ± 0.37	49.96 ± 0.21	+15.22	38.20 ± 0.35	−23.54
	b	48.68 ± 0.18	49.88 ± 0.24	+2.46	43.00 ± 0.21	−13.79
	c	48.26 ± 0.23	49.44 ± 0.17	+2.44	36.40 ± 0.30	−26.38
2	*a*	48.46 ± 0.30	55.56 ± 0.25	+14.65	45.60 ± 0.24	−17.93
	b	50.58 ± 0.27	55.38 ± 0.25	+9.48	49.24 ± 0.20	−11.10
	c	50.00 ± 0.25	55.12 ± 0.25	+10.24	45.14 ± 0.31	−18.11
3	*a*	63.66 ± 0.15	67.12 ± 0.15	+5.43	54.14 ± 0.21	−19.34
	b	63.58 ± 0.19	68.22 ± 0.28	+7.30	59.18 ± 0.27	−13.25
	c	63.72 ± 0.16	68.54 ± 0.15	+7.56	55.50 ± 0.29	−19.03
4	*a*	53.86 ± 0.14	59.82 ± 0.17	+11.06	52.94 ± 0.16	−11.50
	b	53.80 ± 0.13	59.88 ± 0.19	+11.30	54.60 ± 0.17	−8.82
	c	54.20 ± 0.16	59.36 ± 0.17	+9.52	46.96 ± 0.14	−20.89
5	*a*	54.12 ± 0.16	59.42 ± 0.18	+9.79	52.82 ± 0.16	−11.11
	b	53.66 ± 0.13	58.70 ± 0.14	+9.39	55.20 ± 0.14	−5.96
	c	53.78 ± 0.16	59.24 ± 0.17	+10.15	47.20 ± 0.18	−20.33
6	*a*	54.16 ± 0.14	59.82 ± 0.18	+10.45	52.54 ± 0.16	−12.17
	b	53.84 ± 0.15	59.20 ± 0.15	+9.95	55.54 ± 0.16	−6.18
	c	53.62 ± 0.16	58.92 ± 0.15	+9.88	46.80 ± 0.12	−20.58

For meaning of abbreviations, see Table I.

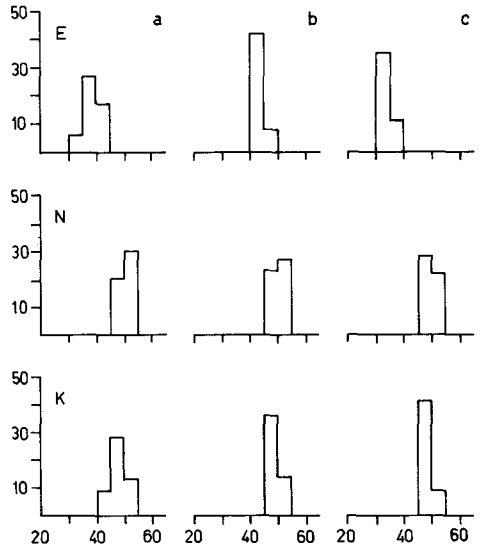

FIGURE 2 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 100 hr of exposure to cold (subgroup 1). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

medulla cells as far as the variations of DNA are concerned.

MATERIAL AND METHODS

A. *Animal Technique*

Adult male and female rats of the Italico strain, bred in our department, fed a standard diet, and weighing about 200 g, were used. Each day they were exposed to cold (+4°C) for 15 hr, followed by 9 hr at room temperature (+18–20°C) as in previous experiments (1–4) (on Sunday the animals were exposed to cold for 20 hr followed by 4 hr at room temperature).

The experimental animals were divided into 9 groups, which were exposed to cold for a total of 50, 100, 200, 300, 350, 400, 500, 600, and 750 hr, respectively. Each group was, in turn, divided into 3 to 6 subgroups, each made up of 6 littermates of the same sex, 3 of which were used as controls.

At the end of the specified cold exposure, each subgroup was treated in the following way: of the experimental animals, one was killed immediately, the second after 9 hr at room temperature, the third was kept at room temperature for 9 hr, then in the cold for 15 hr and killed immediately thereafter.

TABLE III

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 200 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	40.22 ± 0.18	47.20 ± 0.19	+17.35	37.78 ± 0.21	−19.96
	b	40.18 ± 0.18	44.80 ± 0.23	+11.50	38.60 ± 0.47	−13.84
	c	40.62 ± 0.23	46.52 ± 0.20	+14.52	30.96 ± 0.32	−33.45
2	*a*	44.76 ± 0.16	51.74 ± 0.15	+15.59	35.44 ± 0.13	−31.50
	b	44.92 ± 0.14	52.30 ± 0.20	+16.43	38.90 ± 0.23	−25.62
	c	44.80 ± 0.18	51.40 ± 0.22	+14.73	33.52 ± 0.19	−34.79
3	*a*	50.68 ± 0.23	61.14 ± 0.25	+20.64	46.40 ± 0.20	−24.11
	b	57.60 ± 0.24	61.53 ± 0.29	+6.82	52.00 ± 0.26	−15.49
	c	60.86 ± 0.29	62.06 ± 0.27	+1.97	44.14 ± 0.18	−28.88

For meaning of abbreviations, see Table I.

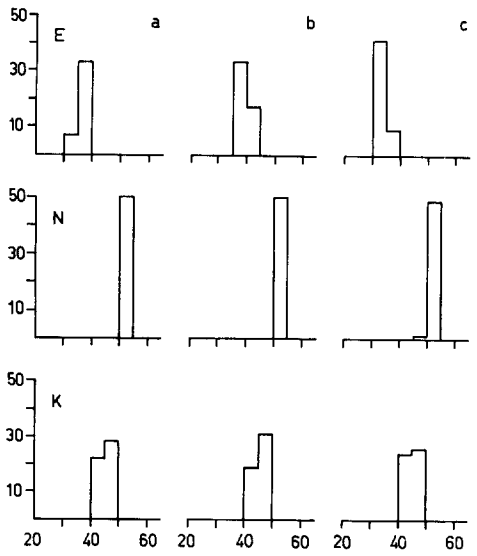

FIGURE 3 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 200 hr of exposure to cold (subgroup 2). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

A control (kept throughout at room temperature) was killed simultaneously with each of the experimental animals.

From each animal both adrenals were dissected out; and, in addition, a piece of kidney was taken from the controls.

A total of 192 rats was used.

B. *Feulgen Photometry*

One of the suprarenal glands from each animal was fixed in 10% neutral formalin according to the technique described in a previous paper (2). The other suprarenal gland was dissected out, cut semicircularly under a dissecting microscope (2), and the fresh medulla tissue freed from the cortex was smeared on a microscope slide according to the following procedure. The slide was divided into three sectors: in one the experimental medulla was smeared, in the second the kidney, and in the third the control medulla. The smears were fixed in 95% ethanol for 10 min. Both smears and sections (12 μ thick) were stained according to the Feulgen method (9). The hydrolysis was carried out in 1 N trichloroacetic acid and the time was 12 min. Preparations of the same subgroup were stained simultaneously.

In each case, on fifty nuclei of the experimental medulla and the control medulla and of the kidney the Feulgen dye content was measured by an integrating histophotometer (10).

C. *Radioautography*

Animals belonging to all the groups exposed to cold, with the exception of those exposed for 50 hr and 200 hr, were examined with this technique. H_3-thymidine (Radiochemical Center, Amersham, England, specific activity 14.8 c/mmole) dissolved in H_2O was injected intraperitoneally in a dose of 1 μc/g body weight. The injections were made at the beginning of the recovery or cold exposure period. Only in the case of the 100-hr cold treatment was H_3-thymidine injected after 6 hr of exposure to room temperature.

TABLE IV

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 300 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	55.30 ± 0.32	60.10 ± 0.28	+8.68	35.84 ± 0.20	−40.37
	b	55.38 ± 0.20	60.10 ± 0.28	+8.52	42.38 ± 0.27	−29.48
	c	51.58 ± 0.26	59.18 ± 0.23	+14.73	33.20 ± 0.30	−43.90
2	*a*	52.46 ± 0.29	60.06 ± 0.23	+14.49	39.82 ± 0.31	−33.70
	b	52.68 ± 0.27	60.16 ± 0.24	+14.20	45.12 ± 0.19	−25.00
	c	53.74 ± 0.19	60.30 ± 0.19	+12.21	32.28 ± 0.19	−46.47
3	*a*	51.50 ± 0.27	59.30 ± 0.24	+15.14	45.32 ± 0.17	−23.58
	b	51.50 ± 0.25	60.26 ± 0.23	+17.00	51.84 ± 0.35	−13.97
	c	51.50 ± 0.23	59.88 ± 0.23	+16.27	41.76 ± 0.21	−30.26

For meaning of abbreviations, see Table I.

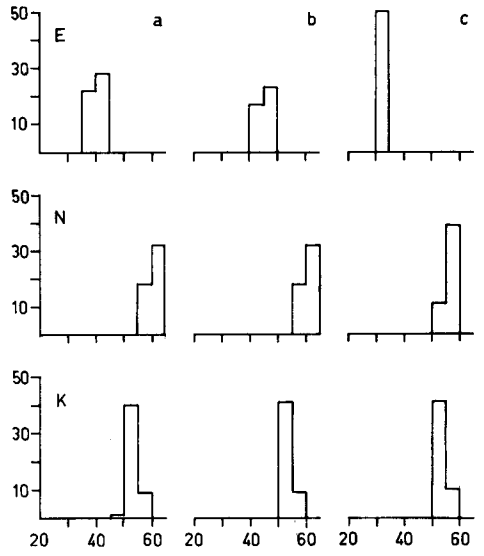

FIGURE 4 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 300 hr of exposure to cold (subgroup 2). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

Fixation, staining, and treatment of the adrenals were the same as described above in paragraph B. The details of the radioautographic technique have been referred to in a previous paper (4). G_5 Emulsion in gel form Ilford (Ilford, England) was used, as well as stripping film (Kodak AR 10), and the exposure time was 10 days in a light-proof box kept at +4°C. The slides were developed with Kodak D 80 for 2 min (emulsion) or 4 min (stripping film), rinsed in a stopping bath, fixed in Kodak Rapid Fixer for 10 min, and then washed in tap water for 1 hr.

From each preparation an average of 8,000 nuclei was scanned, the percentage of the labeled nuclei calculated, and the number of grains per nucleus counted. These values were corrected for the background, which amounted to 1 to 2 grains per nucleus.

RESULTS

A. *Feulgen Photometry*

1. NORMAL ANIMALS: Kidney nuclei are grouped in a single class, with little scatter of values (Figs. 1 to 9). The same is true for the nuclei of the control adrenal medulla, but the average DNA content of these nuclei is 10% higher than that of the kidney nuclei. This fact is in agreement with previous observations (1–4).

2. ANIMALS EXPOSED TO COLD: Also in agreement with previous observations, the amount of DNA per nucleus of the adrenal medulla cells decreases progressively with increasing time of cold exposure: after 50 hr (Table I, Fig. 1) the loss of DNA is 7% of the total, after 100 hr (Table II, Fig. 2) it is 16%, after 200 hr (Table III, Fig. 3) it is 25%, and after 300 hr (Table IV, Fig. 4) the decrease reaches a maximum of 32%.

After 350 hr of cold exposure (Table V, Fig. 5), the DNA lost is reduced to 8%, and in the longer

TABLE V

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 350 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	51.22 ± 0.19	61.60 ± 0.18	+20.26	56.10 ± 0.10	−8.93
	b	53.60 ± 0.19	60.30 ± 0.18	+12.50	63.36 ± 0.16	+5.07
	c	53.72 ± 0.16	61.08 ± 0.15	+13.70	59.50 ± 0.19	−2.59
2	*a*	54.04 ± 0.15	59.72 ± 0.18	+10.51	56.48 ± 0.15	−5.43
	b	54.26 ± 0.12	60.12 ± 0.17	+10.80	64.42 ± 0.14	+7.15
	c	54.44 ± 0.17	60.08 ± 0.16	+10.36	69.02 ± 0.14	+14.88
3	*a*	59.36 ± 0.15	66.88 ± 0.16	+12.67	61.42 ± 0.19	−8.16
	b	60.22 ± 0.18	67.06 ± 0.13	+11.36	73.20 ± 0.14	+9.15
	c	60.64 ± 0.16	66.96 ± 0.13	+10.42	71.28 ± 0.22	+6.45
4	*a*	51.36 ± 0.21	58.64 ± 0.12	+14.17	57.98 ± 0.20	−1.13
	b	52.58 ± 0.14	58.06 ± 0.12	+10.42	60.44 ± 0.19	+4.10
	c	52.04 ± 0.14	58.08 ± 0.13	+11.61	59.24 ± 0.17	+2.00
5	*a*	50.88 ± 0.15	58.86 ± 0.13	+15.68	54.52 ± 0.13	−7.37
	b	51.16 ± 0.17	58.60 ± 0.16	+14.54	60.38 ± 0.18	+3.04
	c	50.26 ± 0.12	58.20 ± 0.13	+15.80	58.40 ± 0.14	+0.34

For meaning of abbreviations, see Table I.

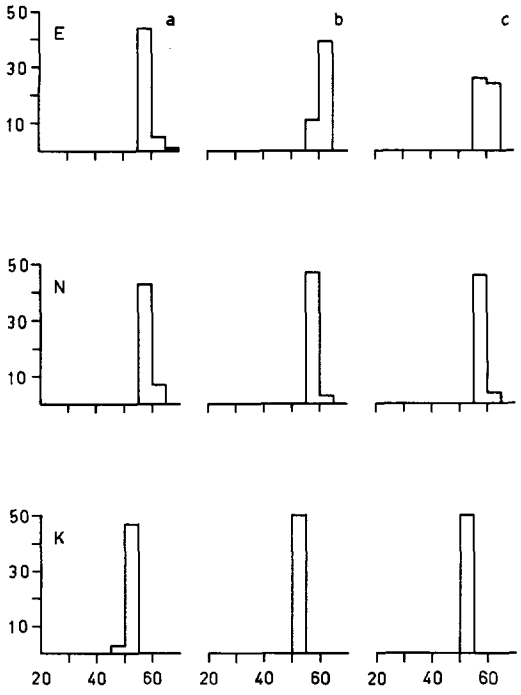

FIGURE 5 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 350 hr of exposure to cold (subgroup 4). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

exposure periods (Tables VI to IX, Figs. 6 to 9) the loss of DNA remains approximately at this level (Fig. 10 *A*).

However, even after 50 hr of exposure to cold) the decrease in amount of DNA (Table I, Fig. 1, is followed by a period of DNA synthesis which takes place at room temperature and by a new loss of DNA during an additional exposure to cold for 15 hr. This phenomenon is even more pronounced after 100, 200, and 300 hr (Tables II to IV, Figs. 2 to 4). The amount of DNA synthesized in each period of recovery (9 hr) increases from 2% after 50 hr of cumulative exposure, to 6% after 100 hr, to 7% after 200 hr, and to 10% after 300 hr (Fig. 10 *B*). On the other hand, the amount of DNA lost in a period of 15 hr at +4°C is 7% after 50 hr of cumulative exposure, 12% after 100 hr, 14% after 200 hr, and 17% after 300 hr (Fig. 10 *B*). It is apparent that the amount of DNA lost is always larger than that synthesized, so that a net cumulative loss results (Fig. 10 *C*).

After 350 hr, the synthesis of DNA during 9 hr at room temperature increases to 12%, and the loss of DNA during 15 hr of cold exposure is reduced to 2% (Fig. 10 *B*). The 24-hr balance is, therefore, positive and an increase of DNA follows (Fig. 10 *C*). This is the cause of the decrease of the loss from 32% at 300 hr to 8%. In the subsequent periods, the synthesis during 9 hr at room

TABLE VI

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 400 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	49.24 ± 0.18	50.38 ± 0.24	+2.31	48.64 ± 0.20	−3.46
	b	44.44 ± 0.10	48.62 ± 0.14	+9.40	47.94 ± 0.07	−1.40
	c	44.50 ± 0.16	54.60 ± 0.11	+22.69	45.46 ± 0.14	−16.74
2	*a*	50.00 ± 0.21	53.48 ± 0.17	+6.96	50.16 ± 0.23	−6.21
	b	41.86 ± 0.24	51.38 ± 0.24	+22.74	54.16 ± 0.28	+5.41
	c	45.40 ± 0.10	54.40 ± 0.23	+19.82	48.68 ± 0.12	−10.52
3	*a*	44.88 ± 0.09	52.60 ± 0.15	+17.20	44.10 ± 0.10	−16.16
	b	47.70 ± 0.13	54.28 ± 0.12	+13.79	58.70 ± 0.09	+8.14
	c	50.06 ± 0.22	54.56 ± 0.17	+8.99	49.50 ± 0.19	−9.28

For meaning of abbreviations, see Table I.

TABLE VII

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 500 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM)	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	43.84 ± 0.20	48.42 ± 0.19	+10.45	44.78 ± 0.22	−7.52
	b	44.00 ± 0.23	48.52 ± 0.22	+10.27	51.38 ± 0.26	+5.89
	c	43.66 ± 0.24	48.34 ± 0.15	+10.71	43.74 ± 0.10	−9.52
2	*a*	44.92 ± 0.18	52.41 ± 0.12	+16.67	45.58 ± 0.18	−13.03
	b	44.60 ± 0.23	50.84 ± 0.18	+13.99	49.74 ± 0.09	−2.16
	c	45.28 ± 0.08	50.82 ± 0.13	+12.23	46.22 ± 0.14	−9.05
3	*a*	48.08 ± 0.28	52.58 ± 0.60	+9.36	44.46 ± 0.41	−15.44
	b	48.86 ± 0.12	55.50 ± 0.33	+13.59	63.84 ± 0.27	+15.03
	c	47.96 ± 0.12	53.12 ± 0.30	+10.76	44.34 ± 0.24	−16.53

For meaning of abbreviations, see Table I.

temperature and the loss during 15 hr of cold exposure both increase.

The amount of DNA lost over a period of 15 hr of cold exposure is 15% after 400 hr, 18% after 500 hr, 19% after 600 hr, and 13% after 750 hr; on other hand, the amount of DNA synthesized during the period of 9 hr at room temperature continues to increase until 500 hr (18%), and afterwards it declines, being 7.5% after 750 hr (Tables VI to IX, Fig. 10 *B*).

Therefore, from 400 hr onwards a steady-state equilibrium is reached in which the loss of DNA taking place in the cold is almost exactly compensated by the synthesis occurring at room temperature.

B. *Radioautography*

The incorporation of H_3-thymidine demonstrated that synthesis of DNA takes place during the recovery period (Table X), in agreement with the previous observations (3, 4) which were carried out at 300 hr of cumulative exposure to cold. A difference has been found in the time of onset of label incorporation into the DNA. After

TABLE VIII

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 600 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears.

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	51.54 ± 0.25	61.02 ± 0.18	+18.39	51.00 ± 0.16	−16.42
	b	53.86 ± 0.21	62.04 ± 0.15	+15.19	60.66 ± 0.23	−2.23
	c	51.98 ± 0.16	61.54 ± 0.18	+18.39	51.12 ± 0.27	−16.93
2	*a*	56.22 ± 0.12	62.80 ± 0.13	+11.70	58.76 ± 0.14	−6.43
	b	55.88 ± 0.21	61.00 ± 0.23	+9.16	73.30 ± 0.25	+20.16
	c	56.54 ± 0.22	64.52 ± 0.17	+14.11	56.72 ± 0.30	−12.09
3	*a*	52.72 ± 0.14	58.24 ± 0.13	+10.47	55.68 ± 0.18	−4.40
	b	53.12 ± 0.13	58.34 ± 0.16	+9.83	58.20 ± 0.15	−0.24
	c	52.34 ± 0.15	58.68 ± 0.14	+12.11	53.10 ± 0.12	−9.51

For meaning of abbreviations, see Table I.

TABLE IX

Variations of Feulgen Dye/Nucleus (Arbitrary Units) Determined by Histophotometry in Adrenal Medulla of Rats after 750 Hr of Cumulative Exposure to Cold

All values are averages of 50 nuclei on smears

Subgroup No.		Kidney (± SEM) K	Normal adrenal medulla (± SEM) N	$\frac{N \times 100}{K}$	Experimental adrenal medulla (± SEM) E	$\frac{E \times 100}{N}$
1	*a*	50.82 ± 0.20	49.26 ± 0.16	−3.0	51.76 ± 0.22	+5.07
	b	51.70 ± 0.20	51.98 ± 0.20	+0.54	57.58 ± 0.14	+10.77
	c	51.72 ± 0.17	51.36 ± 0.16	−0.07	50.30 ± 0.15	−2.06
2	*a*	47.36 ± 0.16	49.50 ± 0.14	+4.52	51.50 ± 0.19	+4.04
	b	47.30 ± 0.15	48.98 ± 0.17	+3.55	55.96 ± 0.17	+14.25
	c	47.14 ± 0.13	49.22 ± 0.17	+4.34	46.62 ± 0.16	−5.28
3	*a*	51.62 ± 0.17	57.66 ± 0.10	+11.70	51.48 ± 0.17	−10.72
	b	52.10 ± 0.16	58.48 ± 0.13	+12.24	54.90 ± 0.14	−6.12
	c	51.60 ± 0.15	57.94 ± 0.13	+12.28	49.80 ± 0.14	−14.05

For meaning of abbreviations, see Table I.

100 hr of exposure to cold, DNA synthesis is detected only after 6 hr of recovery (Table X). Beyond 300 hr, the synthesis begins as soon as the animals are brought back to room temperature. The percentage of labeled nuclei is 1.1% at 300 hr, 7% at 350 hr, and 3.6% from 400 to 750 hr (Table X).

Marked differences in the labeling pattern of the nuclei were also observed when the precursor was administered during the period of cold exposure. As was previously shown (3, 4), no labeling of nuclei takes place during the exposure to cold after 100 and 300 hr, whereas from 350 hr onwards incorporation occurs also during the period of exposure to low temperature.

In general, the number of grains per nucleus is small, indicating a low rate of DNA synthesis (Fig. 11). The percentage of labeled nuclei is higher after 350 hr of exposure to cold than at the subsequent periods. In fact, at this interval the DNA loss during the 15 hr of cold exposure is reduced from 7% at 300 hr to 2%. At 400 to 500 hr the percentage of labeled nuclei is lower, and the reduction in the decrease of DNA is also smaller.

Therefore, the decrease in the DNA content

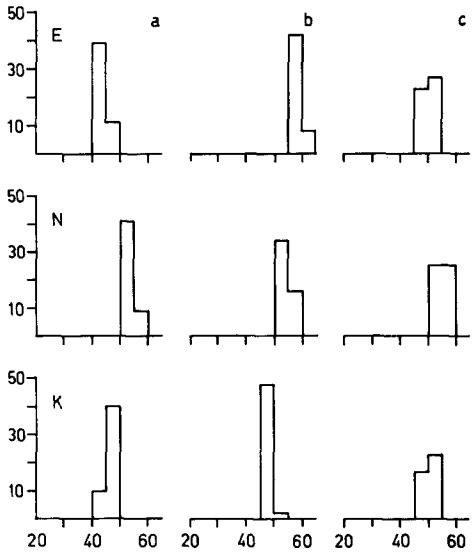

FIGURE 6 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 400 hr of exposure to cold (subgroup 3). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

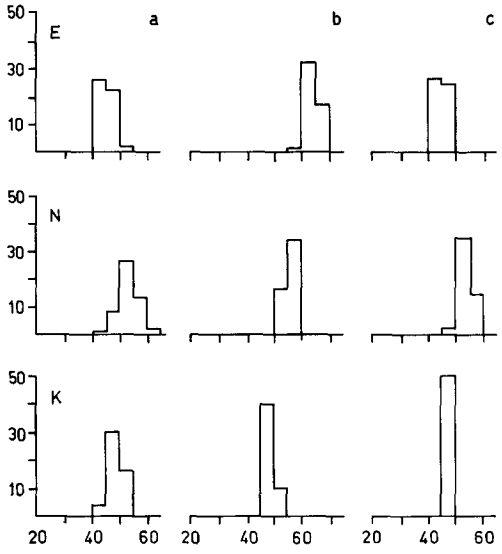

FIGURE 7 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 500 hr of exposure to cold (subgroup 3). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

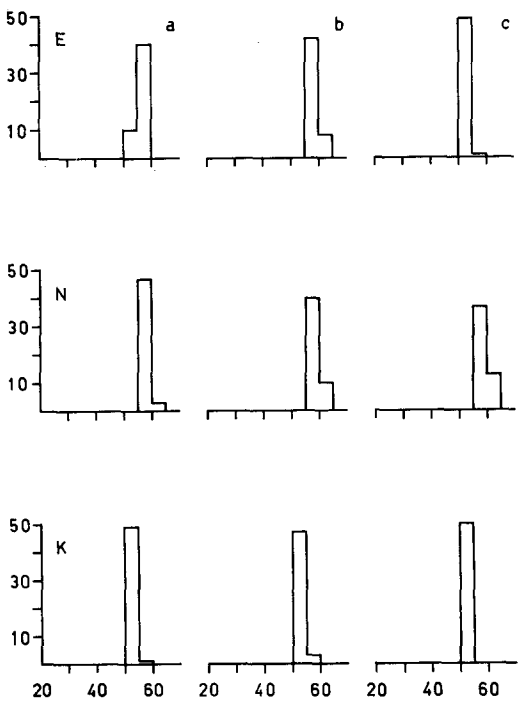

FIGURE 8 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 600 hr of exposure to cold (subgroup 3). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

observed histophotometrically over a period of 15 hr after 350 to 750 hr of cold exposure results from the balance between synthesis and loss taking place simultaneously.

The low percentage of labeled nuclei does not apparently agree with the histophotometric measurements. The reason for this discrepancy is to be found in the short availability time of the labeled precursor (less than 1 hr according to Hughes et al. (11), Quastler and Sherman (12), Koburg and Maurer (13)) and the brief exposure time of the slides (10 days).

In a previous paper (4) it was shown that by increasing the number of injections of H_3-thymidine from 1 to 4, evenly spaced over 8 hr of the recovery period, the percentage of labeled nuclei increased more than proportionately, thus indicating an inhomogeneity in the timing of DNA resynthesis among the various nuclei. Furthermore, if the exposure time of the slides was extended from 10 to 30 days, three times as many

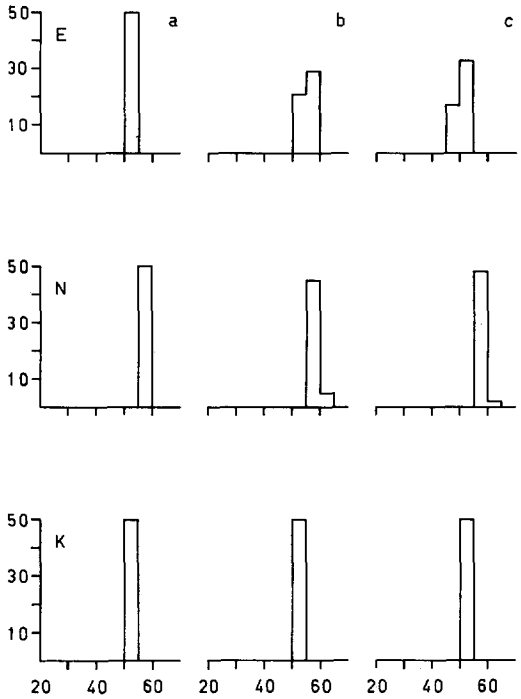

FIGURE 9 Histograms of Feulgen dye content of the nuclei of adrenal medulla after 750 hr of exposure to cold (subgroup 3). For meaning of letters *K*, *N*, *E*, and *a* to *c*, see Fig. 1.

Abscissa, Feulgen dye in arbitrary units; ordinate, number of nuclei measured.

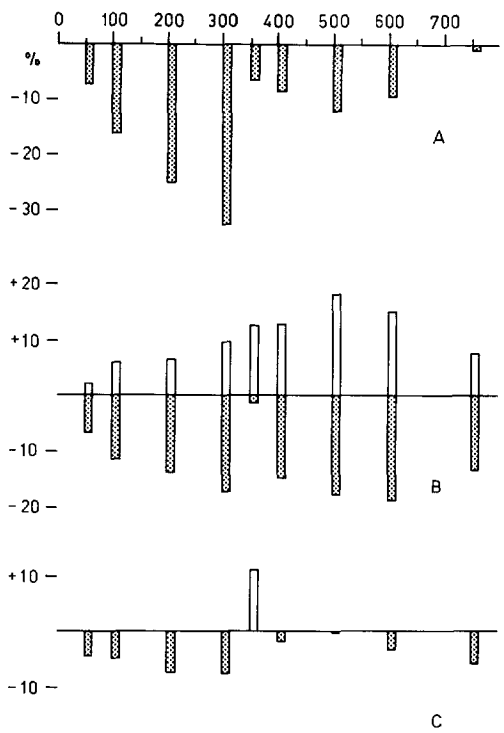

FIGURE 10 *A*, Decrease of DNA/nucleus (as per cent of normal) at the end of the specified cumulative cold exposure. *B*, Loss of DNA during 15 hr of cold exposure (black column) and synthesis of DNA during 9 hr of room temperature (white column) at the end of the specified cumulative cold exposure, expressed as per cent of normal. *C*, Net variations of DNA content (as per cent of normal) at the end of a stimulation cycle (24 hr) after the indicated period of cumulative exposure.

labeled nuclei were found. This shows that many nuclei are so weakly labeled that they cannot be detected by radioautography after the shorter exposure time of 10 days. Taking into account both these factors, and the variations in the extent of the over-all DNA resynthesis among the various nuclei, as shown by Feulgen photometry, it was concluded (4) that there was actually a good quantitative agreement between the histophotometric and radioautographic results in this experimental situation. The DNA synthesis, therefore, takes place during the recovery period in all nuclei, but at different times and at different rates. This can explain the spread of photometric values for the content of DNA of the adrenal medulla nuclei after 10 days of recovery (Fig. 5 *D* in reference 2).

DISCUSSION

The data reported in this paper are in full agreement with those already published, i.e. the DNA content of the adrenal medulla nuclei decreases progressively during exposure to cold, reaching the minimum after 300 hr of cold exposure, and increases during recovery.

A strict quantitative interpretation of these data is prevented by two factors: (1) the relatively small number of experiments performed for each time interval, because of the necessity of exploring as many time intervals as possible; and (2) the variability of the data, which arises because the relatively small variations of DNA are measured on short time intervals (9 hr and 15 hr, respectively) and, above all, because the observations were carried out on different animals, even though littermates, which show a fairly high variability of response to the experimental stimuli. However, some trends emerge which allow explanation of the extent of the total DNA loss observed at the end of the various periods of cumulative exposure.

TABLE X

Labeling of the Adrenal Medulla Nuclei after Intermittent Exposure to Cold

In parenthesis the number of the animals. The numbers of the labeled nuclei are corrected for the background (excluding all the nuclei labeled with grain number equal to or less than that of background).

Time of cumulative exposure at +4°C	Incorporation during exposure to cold			Incorporation during recovery		
	No. of nuclei counted	No. of labeled nuclei	Percentage of labeled nuclei	No. of nuclei counted	No. of labeled nuclei	Percentage of labeled nuclei
100	16,000 (2)	—	—	20,000 (3)	210	1.05*
300	16,000 (2)	—	—	14,000 (3)	116	0.83
350	24,000 (4)	1,985	8.20	26,000 (5)	1,880	7.23
400	24,000 (3)	950	3.95	24,000 (3)	572	2.38
500	12,000 (2)	386	3.22	24,000 (3)	1,036	4.32
600	16,000 (2)	464	2.90	16,000 (2)	471	2.94
750	24,000 (3)	1,583	6.59	24,000 (3)	1,499	6.24

* H_3-thymidine injected after 6 hr of recovery. In all the other cases, H_3-thymidine was injected at beginning of the recovery or of the cold exposure period.

The results show that the decrease of DNA begins early, and is already evident to a limited extent after 50 hr of cumulative exposure to cold. The decrease goes on thereafter, reaching the maximum after 300 hr (average decrease, 32.3%). By increasing the time of exposure to cold, the DNA decrease becomes considerably smaller beginning at 350 hr and settles on to a plateau.

The histophotometric analysis of the quantitative variations of DNA during a period of exposure to room temperature (9 hr) and in the subsequent period of cold exposure (15 hr) at various intervals from the beginning of the experimental period, and the parallel study, under the same conditions, of the DNA synthesis by means of radioautography with H_3-thymidine, give some indications about the mechanisms involved in the DNA decrease observed. It is evident that the variations of the DNA per nucleus are the result of the algebraic summation of two opposing trends which are present with different intensities during the experimental period. They are: the net DNA decrease during the period of cold exposure, and the DNA synthesis during the period of exposure to room temperature. The difference between these two trends increases progressively with cumulative exposure to cold up to 300 hr. This depends on a progressive increase of the DNA loss during cold exposure and an accompanying slower increase of DNA synthesis at room temperature. This fact explains the progressive decrease of the amount of DNA per nucleus in the adrenal medulla cells observed until 300 hr of cumulative exposure to +4°C.

At 350 hr the DNA synthesis takes place also during the 15 hr of exposure to cold. This fact is demonstrated by the results of H_3-thymidine incorporation experiments which show a high percentage of labeled nuclei. As a consequence, the DNA decrease after a 15-hr period of cold exposure changes from 17% at 300 hr to 2% at 350 hr.

Therefore, the 24-hr DNA balance is positive and accounts for a sharp decrease in the DNA loss from 40 to 8%.

The radioautographic data confirm the histophotometric results because they show that the observed DNA increases are really due to DNA synthesis de novo. H_3-thymidine is not incorporated into DNA after 100 hr of cold exposure when it is injected into animals immediately at the end of the exposure to cold. However, incorporation does occur when the precursor is injected after the animal has been returned to room temperature for 6 hr. After 300 hr, the labeled precursor is incorporated also when it is injected immediately at the end of the cold exposure. After 350 hr of cumulative exposure to cold, H_3-thymidine is incorporated also during the cold exposure, i.e. when the net amount of DNA decreases. Thus it is clear that the DNA synthesis which is present only during the recovery period in the first stages of the experiment takes place also during the cold exposure in the successive periods.

This fact accounts for the decrease of the loss of DNA during cold exposure and explains the higher DNA level of the plateau reached after 350 hr

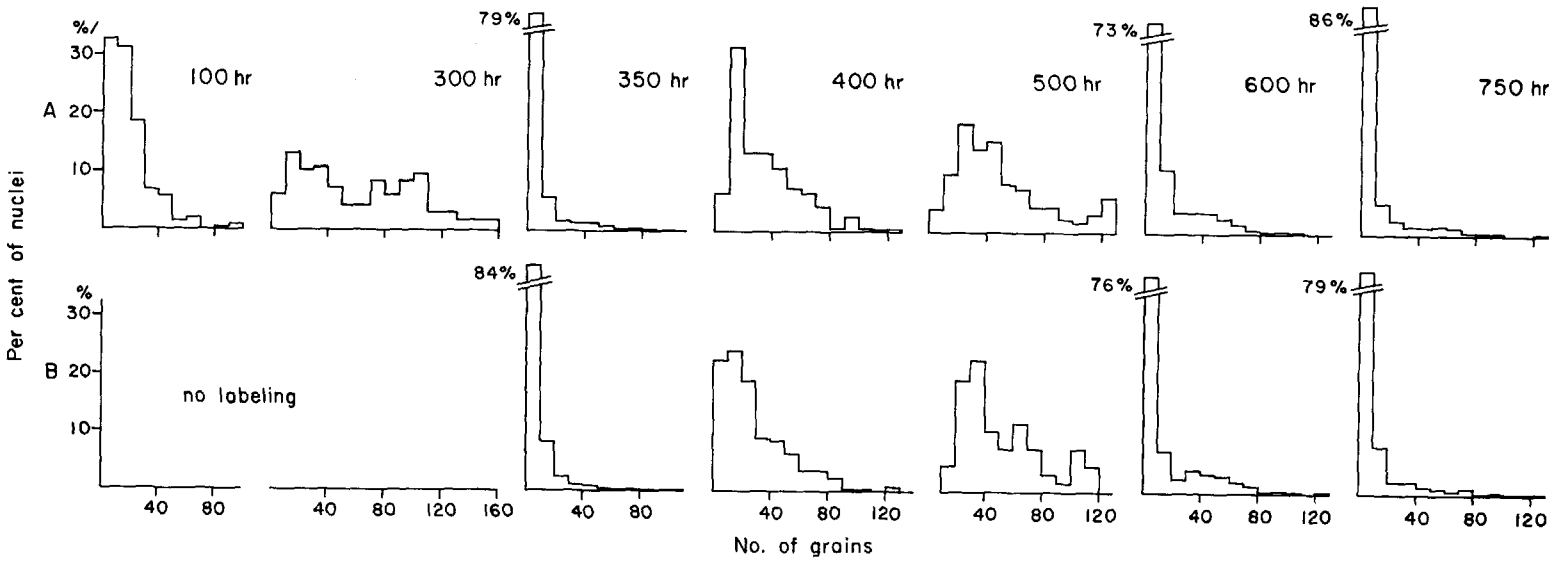

FIGURE 11 Histograms of the distribution of the label in the nuclei of adrenal medulla cells exposed intermittently to cold for 100, 300, 350, 400, 500, 600, and 750 hr. The data are corrected for the background and are expressed as percentage of the total number of labeled nuclei examined. *A*, Labeling during 9 hr at room temperature. *B*, Labeling during 15 hr of exposure to cold.

with respect to the level reached after 300 hr of cold exposure.

Moreover, the fact that incorporation of H_3-thymidine takes place in the period of DNA loss demonstrates that the adrenal medulla cells adapt themselves to maintain, even under the stimulus, the ability to synthesize DNA. This may be relevant to the results obtained by Woodard et al. (14) on cold-treated *Trillium* chromosomes. These authors were unable to demonstrate any DNA loss, despite morphological changes that would suggest it. This result may mean either that no changes in the chromosomal DNA take place, or that a simultaneous breakdown and resynthesis of DNA occurs during the cold treatment, as found in the present work.

It is evident that a process of adaptation takes place in these cells with regard to the experimental situation. This adaptation is a dynamic one, since the cells continue to respond to the stimulus, as shown by the DNA loss during the cold exposure. The loss is quickly compensated for by active processes of synthesis, so that in practice no net loss appears. We may conclude that the adaptation is due mainly to the processes responsible for DNA synthesis.

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BIBLIOGRAPHY

1. VIOLA, M. P., *Nature*, 1964, **204**, 1094.
2. VIOLA-MAGNI, M. P., *J. Cell Biol.*, 1965, **25**, 415.
3. VIOLA-MAGNI, M. P., *Experientia*, 1965, **21**, 716.
4. VIOLA-MAGNI, M. P., *J. Cell Biol.*, 1966, **28**, 9.
5. CRAMER, W., Fever Heat Regulation, Climate and the Thyroid-Adrenal Apparatus, London, Longmans, Green and Co. Ltd., 1928, 31.
6. WALKER, P. M. B., and RICHARDS, B. M., *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York and London, Academic Press Inc., 1959, **1**, 91.
7. BRACHET, J., *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York and London, Academic Press Inc., 1961, **2**, 771.
8. HALE, A. J., *J. Path. and Bact.*, 1963, **113**, 317.
9. LEUCHTENBERGER, C., *in* General Cytochemical Methods, (J. F. Danielli, editor), New York, Academic Press Inc., 1958, **1**, 219.
10. BENEDETTI, P. A., and VIOLA-MAGNI, M. P., *J. Scient. Instr.*, 1966, **43**, 141.
11. HUGHES, W. L., BOND, V. P., BRECHER, G., CRONKITE, E. P., PAINTER, R. B., QUASTLER, H., and SHERMAN, F. G., *Proc. Nat. Acad. Sc.*, 1958, **44**, 476.
12. QUASTLER, H., and SHERMAN, F. G., *Exp. Cell Research*, 1959, **17**, 420.
13. KOBURG, E., and MAURER, W., *Biochim. et Biophysica Acta*, 1962, **61**, 229.
14. WOODARD, J., GOROVSKY, M., and SWIFT, H., *Science*, 1966, **151**, 215.