II. DIFFERENCES IN ADRENAL MEDULLA NUCLEAR DNA CONTENT AMONG RATS OF DIFFERENT STRAINS FOLLOWING INTERMITTENT EXPOSURE TO COLD

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ABSTRACT

The amount of DNA per nucleus in the adrenal medulla cells of four different strains of rats (Wistar, Sprague-Dawley, Long-Evans, and Italico) is determined both under control conditions and after 300 hr of intermittent exposure to cold. The adrenal medulla nuclei of the four strains of rats contain the same amount of DNA; however, the loss of DNA observed after the same experimental treatment differs markedly in the different strains. The loss is small in Wistar and Sprague-Dawley rats (8–13%), larger in Long-Evans rats (20%) and still larger in Italico rats (45%). The DNA loss in Wistar rats increases if the animals are fed the same diet as the Italico rats, and the DNA loss in Italico rats is reduced if the animals are fed the same diet as the Wistar rats. The different behavior of the four strains is discussed in terms of turnover of DNA.

INTRODUCTION

Previous studies (1, 2) have shown that in the adrenal medullary cells of Albino rats of the Italico strain the amount of DNA per nucleus decreases after intermittent exposure to low temperature. The decrease (up to 40% after 300 hr of intermittent exposure to cold) has been ascertained by three different methods, namely: i) histophotometry of the Feulgen dye-DNA complex; ii) microinterferometry (the DNA content per nucleus was calculated by measuring the difference in the dry mass of the nucleus before and after digestion with DNase); iii) microchemical determination of DNA (by diphenylamine reaction) combined with the count of the nuclei in the homogenates. It has also been shown (3) that the loss of DNA is followed by an incorporation of thymidine-^{3}H in the nuclei, indicating synthesis of DNA, when the animals are transferred to room temperature. These two facts strongly suggest that at the end of a given period of cumulative exposure to cold the decrease of the DNA content per nucleus is due to the balance between the loss of DNA occurring during cold exposure and the DNA synthesis which takes place upon return to room temperature (4).

Recently, Cohn and van Duijn (5) and Arold and Sandritter (6) were unable to find any decrease of Feulgen dye-DNA complex in the adrenal medulla nuclei of cold-exposed rats belonging to the inbred strain RT of the Netherlands Cancer Institute and those belonging to the Wistar and Italico strains.

One possible explanation for this discrepancy may be that for some reason the loss of DNA

TABLE I

Number of Animals of Different Strains Used for a) Microinterferometric and b) Biochemical Determinations and Their Experimental Conditions

Strains	Randoin and Causeret's diet		Zoofarm standard diet	
	No. of animals exposed to cold	No. of animals kept at room temp.	No. of animals exposed to cold	No. of animals kept at room temp.
a)				
Italico	14	16	15	6
Long-Evans	11	7	—	—
Sprague-Dawley	—	—	11	6
Wistar	13	9	21	6
b)				
Italico	15	10	—	—
Wistar	—	—	10	10

during a cold period and resynthesis at room temperature could lead to a null balance, thus escaping Feulgen-photometric determinations. In order to test this hypothesis the behaviour of DNA in the adrenal medulla nuclei of rats belonging to different strains has been investigated.

Since the decrease of DNA at the end of 300 hr of intermittent exposure to cold was different in the examined strains, an attempt has also been made to identify some of the possible factors responsible for different losses in different strains.

The present paper is concerned with the comparison of net losses of DNA content per nucleus in adrenal medulla of different strains of rats fed different diets. DNA synthesis investigated by radioautography will be dealt with in a later paper.

MATERIALS AND METHODS

Albino rats of Italico, Wistar, Sprague-Dawley, and Long-Evans strains were used. The animals of the different strains were fed different diets (Randoin and Causeret, reference 7; Zoofarm standard diet Padova, Italy) as shown in Table I. Both Italico and Wistar rats were divided into two groups, one fed with Randoin and Causeret's diet and the other with Zoofarm standard diet. Each group of animals belonging to a given strain (Table I) was further subdivided into two groups. The experimental groups of rats were kept at +2 − 4°C for 15 hr a day and at +20 − 22°C for the remaining 9 hr of the day, over a total period of 20 days (300 hr of total exposure to cold); the control groups of rats, made up of littermates of the animals belonging to the experimental groups, were kept at room temperature (+20 − 22°C) for a period of 20 days. At the end of 300 hr all rats were sacrificed, and the amount of DNA per nucleus of the adrenal medulla cells was determined by means of a microinterferometric technique (8).

Biochemical determinations of DNA per nucleus were also performed in experimental and control groups of Wistar rats kept on Zoofarm standard diet and of Italico rats kept on Randoin and Causeret's diet (Table I *b*).

Microinterferometric Measurement of DNA per Nucleus

The amount of DNA per nucleus was calculated by measuring the difference between the total dry mass of the nuclei (as determined with the microinterferometric method) before and after DNase digestion (8). The adrenal medulla was carefully separated from the cortical tissue (2), homogenized in Barnes, Esnouf, and Stocken's solution (9) with a glass microhomogenizer (Potter-Elvehjem, A. H. Thomas Co. Philadelphia 5, Pa.), and washed with the same solution in a centrifuge (1,100 *g* for 5 min) refrigerated at 0°C. The sediment was resuspended in the medium (9) and divided in three fractions: the first was not subjected to any further treatment; the second was incubated at 37°C for 30 min with an equal amount of medium (9) containing DNase (500 μg/ml final conc.); and the third was treated as the second except that the DNase had been previously inactivated by heating at 90°C for 10 min at pH 5 (10). At the end of incubation at 37°C, cold medium (9) was added to the second and third fractions of the homogenate which were thereafter centrifuged at 1,100 *g* for 5 min. The sediment was washed again with cold medium (9).

In some experiments the adrenal medullas were homogenized in 70% glycerol (11) in order to avoid the loss of soluble material from the nuclei. The homogenate was submitted to the same procedure as that described above; DNase previously dissolved in the medium (9) was added to anhydrous glycerol (30:70, v/v).

The dry mass of the nuclei of the three fractions was determined by microinterferometry. The values obtained from the first fraction represent the dry mass of the materials retained in the nuclei after homogenization and washing. The values of the second fraction represent the materials left in the nuclei after DNase extraction and incubation. The values of the third fraction give a measure of the nuclear materials left in the nuclei after incubation alone.

An integrating microinterferometric apparatus (12) was used. This apparatus determines the surface integral of the light intensities transmitted through

TABLE II

Average Amount of Nuclear Dry Mass Extracted by DNase Treatment from the Nuclei of Adrenal Medulla Cells of Different Strains of Rats Under Normal and Experimental Conditions

The values are expressed in g $\times 10^{-12} \pm$ S.E.M.

Strains	Control	Experimental	Significance of the difference between the means	
			t	*P*
Italico	9.6 ± 0.1 (9)	5.5 ± 0.2 (9)	21.808	>0.001
Long-Evans	9.6 ± 0.04 (6)	7.3 ± 0.1 (9)	13.836	>0.001
Sprague-Dawley	9.5 ± 0.1 (4)	8.2 ± 0.1 (9)	5.661	>0.001
Wistar	9.6 ± 0.1 (4)	8.8 ± 0.1 (8)	3.941	0.01 < P <0.001

In parentheses the number of the experiments performed.

two fields, one of which, of known area, is used as a "measuring field" and the other is used as a "reference field". When the light intensities of the two fields are matched and then an object is moved into the measuring field, a change of light intensity occurs. This change is a function of the total optical path difference (OPD) of the object times the ratio object area/measuring field area. When the light intensities of the fields are matched again by an adequate compensation with the goniometer analyzer, the measure of the OPD of the object integrated over the area of the measuring field is obtained. The error affecting the total OPD measurements is small if the maximum OPD of any point of the object does not exceed 90° (12); in fact, it can be calculated that the error involved in measuring the optical retardation of the nuclei examined is about ±2% (8, 12).

The dry mass (M) was calculated by the equation (8):

$$M = \frac{2\,x^\circ\,\lambda}{360} \cdot \frac{A}{100\,\alpha} \qquad [1]$$

where x° is the half of the nuclear integrated OPD times the ratio nucleus area/measuring field area; λ is the wavelength of the monochromatic light used ($= 546$ mμ); A is the area of the measuring field, and α is the specific refractometric increment (12).

The α values used for the nuclei both before and after DNase treatment examined in Barnes et al. (9) solution and in 70% glycerol were 0.00180 and 0.00103, respectively. The reasons for this choice as well as for the use of the same value of α for nuclei before and after DNase digestion have been discussed elsewhere (8).

Biochemical Determinations

The adrenal medullas, dissected as previously described (2), were homogenized in Barnes et al. solution (9) with a Potter-Elvejhem microhomogenizer. The homogenate was centrifuged at 1,100 *g* for 5 min at 0°C and the sediment was resuspended in 0.25 N perchloric acid and used for biochemical determinations of the DNA according to the micromethod of Steele, Sfortunato, and Ottolenghi (13). The procedure of Steele, Okamura, and Busch (14) was used to remove lipids, and the determination of the DNA was performed by the diphenylamine reagent according to the method of Burton (15).

The amount of DNA per nucleus was calculated by dividing the total DNA amount by the number of nuclei present in the homogenate according to the method described in a previous paper (2).

RESULTS

DNA Content per Nucleus in Control Animals

MICROINTERFEROMETRIC DETERMINATIONS: The amount of DNA calculated by the difference between the dry mass of the nuclei before and that after DNase treatment is the same in the adrenal medulla nuclei of the rats of Italico, Wistar, Sprague-Dawley, and Long-Evans strains, irrespective of the diet.

After DNase treatment at 37°C the nuclei lose $9.5 - 9.6 \times 10^{-12}$ g (Table II). This loss of material is produced as a consequence not only of the action of DNase but also of: i) the action of other enzymes, which may possibly contaminate the DNase; ii) activation of endonuclear enzymes produced by the incubation at 37°C, and iii) the treatment. Therefore, the amount of material lost from the nuclei as a consequence of DNase action can be calculated by subtracting the amount of material lost after treatment at 37°C with inactivated DNase from the total amount of ma-

TABLE III

Amount of DNase-Sensitive Material per Nucleus, Determined by Microinterferometry and Expressed in g × 10^{-12} ± S.E.M., in the Adrenal Medulla of Rats of Different Strains (Aqueous nuclei)

In each experiment, pooled nuclei from 6 adrenal medullas were used. Each value represents the average of 20 nuclei.

Strains	No. exp.	A*	B	C	D	E	F
Italico	1	25.56 ± 0.51	15.84 ± 0.22	9.72	23.94 ± 0.41	1.62	8.10
	2	25.26 ± 0.40	15.84 ± 0.38	9.42	24.18 ± 0.48	1.08	8.34
Long-Evans	1	27.41 ± 0.49	17.76 ± 0.41	9.65	26.16 ± 0.35	1.25	8.40
	2	29.46 ± 0.68	19.86 ± 0.60	9.60	28.14 ± 0.44	1.32	8.28
Sprague-Dawley	1	28.44 ± 0.42	18.78 ± 0.34	9.66	27.06 ± 0.40	1.38	8.28
	2	28.68 ± 0.64	19.02 ± 0.53	9.66	27.36 ± 0.43	1.32	8.34
Wistar	1	29.10 ± 0.61	19.32 ± 0.47	9.78	27.76 ± 0.57	1.44	8.34
	2	29.70 ± 0.52	20.28 ± 0.44	9.42	28.68 ± 0.38	1.02	8.40

*A, dry mass of nuclei before treatment with DNase; B, dry mass of nuclei after treatment with DNase; C, total amount of the nuclear dry mass lost by the treatment (37°C × 30 min) and DNase action; D, dry mass of nuclei after incubation with DNase inactivated at 90°C × 10 min; E, amount of the nuclear dry mass nonspecifically lost by the treatment (37°C × 30 min.); F, amount of the nuclear dry mass specifically lost by the DNase action.

TABLE IV

Amount of DNase-Sensitive Material per Nucleus, Determined with Microinterferometry and Expressed in g × 10^{-12} ± S.E.M., in the Adrenal Medulla of Rats of Different Strains Under Normal Conditions and After Intermittent Exposure to Cold (Nuclei suspended in 70% glycerol)

In each experiment, pooled nuclei from 6 adrenal medullas were used. Each value represents the average of 20 nuclei.

Strains	Control					
	A	B	C	D	E	F
Italico	37.80 ± 0.81	27.97 ± 0.74	9.83	36.72 ± 0.65	1.08	8.75
Wistar	41.58 ± 0.86	31.97 ± 0.82	9.61	40.18 ± 0.67	1.40	8.21
	Experimental					
Italico	37.80 ± 0.75	32.18 ± 0.68	5.62	36.50 ± 0.81	1.30	4.32
Wistar	40.39 ± 0.83	31.86 ± 0.71	8.53	38.99 ± 0.93	1.40	7.13

For the meaning of A–F see Table III.

terial lost after DNase treatment. The results are thus corrected for the loss of material occurring independently of DNase action. The amount of DNase-extractable material corresponds to 8.1 − 8.4 × 10^{-12} g, with an average of 8.3 × 10^{-12} g (Table III). The DNase-extractable material of the nuclei of kidney tubules has been evaluated by the same method and found to be 6.1 × 10^{-12} g (unpublished observation); this amount of DNA per nucleus corresponds closely to that reported in previous findings (16). As discussed in a previous paper (8), it can be concluded that the material lost from adrenal medulla nuclei as a consequence of DNase action alone (8.3 × 10^{-12} g) corresponds closely to the DNA content per nucleus (Table III). Moreover, the amount of DNA per

TABLE V

DNA Content per Nucleus, expressed in g $\times 10^{-12} \pm$ S.E.M., Determined with Biochemical Technique in Normal Animals and in Animals Exposed to Cold

In parentheses the number of experiments performed.

Strains	Animals exposed to cold	Animals kept at room temperature	Percentage decrease	t	P
Italico	5.6 ± 0.55 (3)	8.5 ± 0.45 (2)	34%	3.597	$0.05 < P < 0.02$
Wistar	7.7 ± 0.12 (2)	8.2 ± 0.05 (2)	6%	4.300	$P = 0.05$

TABLE VI

Amount of DNase-Sensitive Material per Nucleus Determined by Microinterferometry and Expressed in g $\times$ $10^{-12} \pm$ S.E.M., in the Adrenal Medulla of Rats of Different Strains Exposed Intermittently to Cold for 300 hr (Aqueous nuclei)

In each experiment, pooled nuclei from 6 adrenal medullas were used. Each value represents the average of 20 nuclei.

Strain	No. exp.	A	B	C	D	E	F
Italico	1	25.34 ± 0.49	19.53 ± 0.33	5.81	24.14 ± 0.41	1.20	4.61
	2	23.16 ± 0.50	17.58 ± 0.35	5.58	22.02 ± 0.47	1.14	4.44
Long-Evans	1	25.86 ± 0.37	18.36 ± 0.39	7.50	24.72 ± 0.42	1.14	6.36
	2	25.26 ± 0.45	17.82 ± 0.37	7.44	24.24 ± 0.44	1.02	6.42
Sprague-Dawley	1	29.94 ± 0.64	21.30 ± 0.44	8.64	28.74 ± 0.41	1.20	7.44
	2	27.18 ± 0.59	18.96 ± 0.28	8.22	25.98 ± 0.44	1.20	7.02
Wistar	1	27.18 ± 0.47	18.48 ± 0.41	8.70	25.80 ± 0.53	1.38	7.32
	2	27.54 ± 0.43	18.54 ± 0.41	9.00	26.58 ± 0.43	0.96	8.04

For the meaning of A–F see Table III.

nucleus determined microinterferometrically in aqueous medium is identical to that determined in 70% glycerol (Table IV). In this medium (glycerol) the total dry mass of the nuclei is larger, since little or no loss of soluble material has occurred.

BIOCHEMICAL DETERMINATIONS: Biochemical determinations of the amount of DNA per nucleus in adrenal medulla cells were performed on Italico and Wistar rats fed respectively with Randoin and Causeret's diet and Zoofarm standard diet. The amount of DNA per nucleus estimated with the biochemical technique is the same in Italico rats and in Wistar rats kept at room temperature, i.e., $8.2 - 8.5 \times 10^{-12}$ g (Table V). This value is in good agreement with that obtained in the present investigations with the microinterferometric technique (see above).

DNA Content per Nucleus in Experimental Animals

MICROINTERFEROMETRIC DETERMINATIONS: After 300 hr of intermittent exposure to cold the amount of DNA per nucleus decreases slightly in Wistar and Sprague-Dawley rats, whereas a clear-cut decrease is observed in Long-Evans rats and an even greater decrease is seen in Italico rats (Tables II, IV, and VI).

In Wistar rats the amount of DNA lost is 0.7×10^{-12} g (equal to 8% of the control), in Sprague-Dawley rats 1.1×10^{-12} g (13% of the control), in Long-Evans rats 1.9×10^{-12} g (23% of the control), and in Italico rats 3.8×10^{-12} g (45% of the control) (Table VII).

The same results have been obtained with

Table VII

Comparison Between the Amounts of DNase-Sensitive Material per Nucleus, Determined with Microinterferometry and Expressed in g $\times 10^{-12} \pm$ S.E.M., in the Adrenal Medulla of Rats of Different Strains Exposed Intermittently to Cold for 300 hr with Respect to the Controls

	Italico	Long-Evans	Sprague-Dawley	Wistar
Control				
1	8.10	8.40	8.28	8.34
2	8.34	8.28	8.34	8.40
Experimental				
1	4.61	6.36	7.44	7.32
2	4.44	6.42	7.02	8.04
Decrease % of experimental with respect to the control	45%	23%	13%	8%

nuclei suspended in 70% glycerol (Table IV). This fact indicates that the decrease of the DNA content cannot be attributed to loss of a soluble fraction of DNA in the aqueous medium.

BIOCHEMICAL DETERMINATIONS: The results obtained with the biochemical technique show that after cold exposure the amount of DNA per nucleus decreases to 5.6×10^{-12} g in Italico rats and to 7.7×10^{-12} g in Wistar rats. These data are in good agreement with those found with microinterferometry; the small discrepancy can be accounted for by the contribution to the total DNA made by connective tissue nuclei, a complicating factor which obviously does not interfere with microinterferometric determinations (Table V).

Effect of Diet on DNA Loss in Wistar and Italico Rats

The amount of DNA per nucleus in control Wistar rats fed Randoin and Causeret's diet and in Italico rats fed Zoofarm standard diet was determined by means of microinterferometric technique. In both strains an amount of 8.2×10^{-12} g of DNA per nucleus was found, a value identical to that of control Wistar and Italico rats kept on Zoofarm and Randoin Causeret's diets, respectively (see above). However, in Wistar rats fed with Randoin and Causeret's diet the decrease of DNA per nucleus after exposure to cold is more marked than that observed in Wistar rats fed with Zoofarm standard diet, and becomes greater in the following generations. After three generations, corresponding to a period of seven months, the amount of DNA per nucleus upon exposure to cold is 4.5×10^{-12} g, a value corresponding to that found in Italico rats fed the same diet, under the same experimental conditions. On the other hand, Italico rats fed with Zoofarm standard diet present a progressively smaller decrease of DNA per nucleus after cold exposure (6.8×10^{-12} g). The value observed after three generations is near to that observed in Wistar rats fed with Zoofarm standard diet, and it is significantly higher than that found after cold exposure in Italico rats on Randoin and Causeret's diet (Table VIII).

DISCUSSION

In our experiments the amount of DNA per nucleus has been evaluated by measuring the difference in the nuclear dry mass before and after treatment with DNase and with a biochemical technique. The reliability of the results obtained with the microinterferometric method is shown by the close agreement with the results obtained with biochemical determinations.

Our experiments show that the amount of DNA per nucleus in the adrenal medulla of rats is 8.3×10^{-12} g in all the strains that we examined (Italico, Long-Evans, Sprague-Dawley, and Wistar). This value is lower than that of 9.5×10^{-12} g obtained by Viola-Magni (2), using a microinterferometric technique.

This discrepancy can be accounted for by the fact that in the previous experiments (2) no correction was made for nonspecific extraction of the nuclei occurring during incubation with the DNase. Higher initial values were obtained therefore, which correspond closely to those found in the present study, if the correction for nonspecific nuclear losses is not taken into account.

After intermittent exposure to cold for a total period of 300 hr the amount of DNA per nucleus decreases. This decrease is small in Wistar and Sprague-Dawley rats (8–13%), whereas in Long-Evans rats the DNA per nucleus decreases by 20% and in the Italico rats the loss is 45%. This last datum is in full agreement with the observations made by Viola-Magni (1, 2) who showed a decrease of 40% of the DNA content of adrenal

TABLE VIII

Effect of Diet Composition on DNA Loss in Wistar and Italico Rats After 300 hr of Intermittent Cold Exposure (Aqueous nuclei)

In each experiment, pooled nuclei from 6 adrenal medullas were used. Each value (in g $\times 10^{-12} \pm$ S.E.M.) represents the average of 20 nuclei.

Generations	A	B	C	D	E	F	G*
			Italico rats fed Zoofarm standard diet				
			Control				
2nd	30.9 ± 0.4	21.4 ± 0.4	9.5	29.5 ± 0.5	1.4	8.1	—
3rd	30.7 ± 0.5	21.2 ± 0.5	9.5	29.4 ± 0.4	1.3	8.2	—
			Experimental				
1st	32.7 ± 0.4	26.3 ± 0.5	6.4	31.3 ± 0.4	1.4	5.0	39%
2nd	32.8 ± 0.5	25.7 ± 0.5	7.1	31.6 ± 0.5	1.2	5.9	28%
	32.5 ± 0.5	25.0 ± 0.3	7.5	31.2 ± 0.4	1.3	6.2	24%
	32.6 ± 0.5	25.0 ± 0.4	7.6	31.3 ± 0.5	1.3	6.3	23%
3rd	29.4 ± 0.4	21.5 ± 0.4	7.9	28.3 ± 0.4	1.1	6.8	17%
			Wistar rats fed Randoin and Causeret's diet				
			Control				
3rd	28.8 ± 0.4	18.8 ± 0.4	10.0	27.1 ± 0.4	1.7	8.3	—
	34.7 ± 0.3	24.6 ± 0.3	10.1	32.8 ± 0.4	1.9	8.2	—
			Experimental				
1st	26.0 ± 0.4	17.8 ± 0.4	8.2	24.7 ± 0.4	1.3	6.9	16%
	24.6 ± 0.3	17.2 ± 0.4	7.4	23.4 ± 0.3	1.2	6.2	27%
2nd	29.6 ± 0.4	22.8 ± 0.6	6.8	28.3 ± 0.5	1.3	5.5	33%
	26.3 ± 0.5	19.4 ± 0.5	6.9	24.6 ± 0.5	1.7	5.2	37%
3rd	31.5 ± 0.2	25.2 ± 0.2	6.3	29.8 ± 0.2	1.7	4.6	44%
	25.9 ± 0.7	20.0 ± 0.6	5.9	24.5 ± 0.6	1.4	4.5	45%
	26.5 ± 0.6	20.6 ± 0.5	5.9	25.0 ± 0.4	1.5	4.4	47%

For the meaning of A–F see Table V.
*G: percentage decrease with respect to the control.

medulla nuclei of Italico rats after a total of 300 hr of intermittent exposure to cold.

It is clear, therefore, that the amount of DNA per nucleus in the adrenal medulla decreases in all four strains of rats under the same experimental conditions, although to a different degree. In two strains the decrease is so small that it is likely to escape detection with Feulgen-photometry, a method which may be affected by many sources of error (17).

Our experiments show clearly that the behaviour of DNA of adrenal medulla cells upon exposure to cold is affected by the diet. The importance of this factor is demonstrated by the fact that reversing the diets between Wistar and Italico rats results in a more conspicuous loss of DNA (up to 45%) in Wistar rats after cold exposure and in a reduced DNA loss (17%) in Italico rats after the same experimental procedure, whereas the amount of DNA per nucleus in normal animals is not affected.

The effect of the diet on DNA behaviour increases, in our experiments, among three generations of animals as if the causative factor (or factors) were slowly "stored" and slowly "released". This problem, together with the questions

concerning the nature and the mechanisms of action of the dietary factors, whether a direct influence on DNA behaviour or only a side-phenomenon of a more direct effect on other organs, must await further experiments.

It is interesting to note that, after three generations of rats fed Zoofarm standard diet, the DNA decrease per nucleus of adrenal medullas of Italico rats after cold exposure is still larger than that of Wistar rats kept on the same diet. Moreover, Long-Evans rats, kept on Randoin and Causeret's diet, show a much smaller DNA decrease per nucleus than Italico rats fed the same diet when exposed to cold. These results may be regarded as an indication that, besides the diet, other factors, such as strain differences, exert an influence on the DNA decrease per nucleus of the adrenal medulla. The importance of the latter factor for the adrenal medulla has been stressed by Coupland (18).

Concerning the decrease of DNA produced by exposure to cold, it has been observed that in Italico rats during each exposure to cold (15 hr a day) the DNA content per nucleus decreases, and then increases again during the successive 9 hr at room temperature (4). However, DNA synthesis does not compensate for loss, so that after 24 hr of experiment the balance is negative. The 40 – 45% decrease of DNA found at the end of 20 days of experiment (300 hr of cumulative exposure to cold) represents the sum of the accumulated negative daily balance.

After a longer cold stimulation (350 hr), synthesis of DNA takes place also during the cold exposure period, and quickly makes up for the loss so that the net amount of DNA is only 10% less than the normal (4).

If it is assumed that the same situation holds true for other strains of rats, the hypothesis could be suggested that the balance between losses and syntheses may be different in the different strains of rats, and may be altered by other factors, such as diet composition.

According to this hypothesis, in Wistar and Sprague-Dawley rats fed with Zoofarm standard diet, both loss and synthesis would proceed simultaneously, with only a slight quantitative preponderance of the loss. After 300 hr, therefore, the amounts of DNA lost would be almost compensated for by synthesis, and only a slight net decrease of DNA would become detectable. This interpretation is fully supported by autoradiographic experiments in which the synthesis of DNA has been investigated in adrenal medulla nuclei of rats belonging to different strains and fed with different diets.[1]

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