Brit. J. Pharmacol. (1950), 5, 604.

THE EFFECT OF METHIONINE AND OF THIOURACIL ON THE METHYLATION OF *NOR*ADRENALINE IN THE BODY

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(Received September 18, 1950)

When insulin is injected into a rat, the result of the hypoglycaemia is a discharge of amines from the adrenal medulla. Burn, Hutcheon, and Parker (1950b) have found that when the hypoglycaemia is prolonged the total quantity of amines which can be extracted from the glands falls, and the proportion of *nor*adrenaline rises. Thus, after the injection of 0.2 unit insulin per 100 g., the total amine content fell from 0.92 mg./g. to 0.44 mg./g. at the end of 8 hr. and the proportion of *nor*adrenaline rose from 10 per cent to 33 per cent at the same time. The explanation suggested for the rise in the proportion of *nor*adrenaline was that, after a prolonged period of amine production and discharge, the medulla manufactured or accumulated *nor*-adrenaline at a faster rate than it was able to methylate it. This explanation assumes that *nor*adrenaline is the precursor of adrenaline; evidence for this has already been obtained by Bülbring (1949), who showed that minced adrenal tissue from cat and from dog was able to convert *nor*adrenaline to adrenaline provided that adenosine triphosphate was present. Bülbring and Burn (1949b) also showed that *nor*-adrenaline is converted to adrenaline in the isolated perfused adrenal gland of the dog.

The percentage of *nor*adrenaline in the adrenal gland of the rat some hours after the injection of insulin can therefore be used as an index of the power of the adrenal gland to perform methylation. We know from the work of Keller, Boissonas, and du Vigneaud (1950) that when methionine, containing labelled carbon in the $-CH_3$ group, is added to the diet of rats, the labelled carbon is found in the $-CH_3$ group of adrenaline isolated from the adrenal gland. The question then arises whether the rate of methylation can be accelerated by adding methionine to the diet. The question has clinical importance because there may be men whose adrenal glands contain a high proportion of *nor*adrenaline. Holtz and Schümann (1950) have found that *nor*adrenaline is present in the normal adrenal medulla of man, but it is not known whether the proportion varies in different subjects as it does in cats. Bülbring and Burn (1949a) found that in cats the proportion liberated by splanchnic stimulation ranged from 2 to 85 per cent of the mixture with adrenaline. If some individuals liberate a large proportion of *nor*adrenaline, then, since this substance has much less bronchodilator action than adrenaline, these individuals might be less able to overcome a bronchospasm, and might on that account be asthmatics. If the rate of methylation could be increased by increasing the amount of methionine in the diet, the administration of methionine might be beneficial to some asthmatics.

The second question to which we have sought to find an answer is whether the thyroid hormone modifies the rate of methylation. In discussing methylation Dr. R. B. Bourdillon referred us to the paper by Stuber, Russman, and Proebsting (1923), who showed that, after removal of the thyroid gland, rabbits were unable to form creatine from guanidoacetic acid. We have therefore compared the percentage of *nor*adrenaline in the adrenal gland some hours after the injection of insulin in normal rats, or rats given thyroxine, with the percentage in rats given thiouracil for some time beforehand.

RESULTS

Rats deficient in methionine.—The effect of methionine deficiency was first studied in rats fed on a basic diet low in methyl-donating compounds. This diet was adapted from Beveridge, Lucas, and O'Grady (1944), who found that it was just sufficient to maintain the body-weight of young adult rats (150–200 g.). One experiment will be described in detail.

Two groups of six " Wistar " rats, mixed males and females between 50–80 g. in wt., were given a diet for 14 days consisting of 8 per cent casein, 12 per cent gelatin, 8 per cent margarine, 4 per cent salt mixture, and 68 per cent sucrose. This diet was made up in bulk and stored in the cold-room. The rats ate about 8.0 g. per day each.

This diet was supplemented in the following way:

Deficient group	*Methionine group*
4.0 g. aneurin	4.0 mg. aneurin
80 μg. riboflavin	80 μg. riboflavin
50 mg. glycine	100 mg. DL-methionine

The supplements were given to each rat every other day by stomach-tube, in the form of aqueous solutions.

After six days on this diet, both groups were kept without food overnight and injected the next morning with 0.4 unit insulin per 100 g. body wt. This was done to deplete the pressor amines in the adrenal medulla and ensure reformation. The rats were given food again the same night, and the diet was continued until the thirteenth day, when they were once more kept without food overnight and injected the next morning with 0.4 unit insulin per 100 g. They were killed 12 hours later, and the adrenal glands extracted in 0.1 N-HCl. The preparation and estimation of these extracts have been described by Burn, Hutcheon, and Parker (1950a). The extracts were injected into a spinal cat to record the rise of blood pressure and the contraction of an innervated nictitating membrane. The injections

TABLE I

ESTIMATION OF PROPORTION OF ADRENALINE IN SUPRARENAL EXTRACT

Obs.	Injection	Rise in B.P. (*b*)	Contraction of nictitating membrane (*a*)	Ratio $\frac{a}{b} \times 1{,}000$
42	0.9 ml. 50%A + 50%N	104 mm.	8 mm.	77
43	0.5 ml. supplemented extract	104	11	106
44	1.0 ml. 75%A + 25%N	106	12	113
45	10 μg. *l*-adrenaline	104	17	163

TABLE II

COMPARISON OF SUPRARENAL EXTRACTS FROM DEFICIENT RATS AND RATS GIVEN METHIONINE

Obs.	Injection	Rise of blood pressure (b)	Contraction of nictitating membrane (a)	Ratio $\frac{a}{b} \times 1,000$
8	0.5 ml. methionine extract	92 mm.	15.5 mm.	168
9	0.5 ml. deficient extract	111	16	144
10	0.5 ml. methionine extract	105	16	152
12	0.2 ml. deficient extract	63	6.5	103
13	0.2 ml. methionine extract	62	8	129
14	0.3 ml. methionine extract	84	12	142
15	0.3 ml. deficient extract	90	12	133
16	0.2 ml. deficient extract	65	8	123
17	0.2 ml. methionine extract	62	9	145

were given between injections of known mixtures of adrenaline and *nor*adrenaline, the amounts of these mixtures and of the extracts being such as to give about the same rise in blood pressure. The figures in Table I show how one estimate of the extract from the methionine-supplemented rats was obtained.

The ratios in Table I were plotted as ordinates against the adrenaline percentage in the known mixtures; this percentage was 50 per cent in obs. 42, 75 per cent in obs. 44, and 100 per cent in obs. 45. The percentage of adrenaline in the " supplemented " extract was then read off from this graph and found to be 70 per cent. Three estimates of each extract were obtained in a similar manner, and the mean result showed that the deficient extracts contained 64 per cent and the methionine-supplemented extracts 74 per cent adrenaline. This is experiment 6 in Table III.

As a further example of the evidence on which the results in Table III are based, the details of the results of Exp. 1 are given in Table II. In this experiment the effects of injecting the extract from the deficient rats were repeatedly compared with the effects of the extract from the rats with a methionine supplement.

When each injection of " deficient " extract in Table II is compared with the adjacent injection of " methionine " extract which had the same or a very similar pressor effect, the ratio for the " deficient " extract was in every case smaller than the ratio for the methionine extract. That is to say, the extract prepared from the methionine-supplemented animals consistently showed a higher proportion of adrenaline than the extract prepared from those which were deficient in methionine.

The results in the six experiments with rats given a deficient diet are summarized in Table III, which shows that in all the experiments a variable but consistently

TABLE III

SUPRARENAL EXTRACTS OF RATS GIVEN DEFICIENT DIET

Type of rat	Adrenaline percentages						
	Exp. 1	2	3	4	5	6	Mean
Deficient rats	80	76	66	52	86	64	70.6
Rats given methionine	93	95	72	59	90	74	80.5

TABLE IV

SUMMARY OF METHIONINE-DEFICIENT DIET EXPERIMENTS

Ch.Cl. = choline chloride; M = DL-methionine (Roche). Nic. = nicotinamide (B.D.H.); CHO/fat = protein-free diet. All rats received 2 mg. aneurin per day and the rats in Exp. 6 also received 40 μg. riboflavin per day

Exp.	Wt. of rats (g.)	Composition of basic diet	Supplements per rat per day		Duration of experi-ment	Insulin per 100 g. wt.	Time of death post-insulin
			Methionine group	Deficient group			
1	70–95	18% casein	24 mg. M.	2.0 mg. Nic.	13 days	0.4 i.u.	4½ hr.
2	55–90	8% casein 12% gelatin	24 mg. M. + 24 mg. Ch.Cl.	2.0 mg. Nic.	14 days	0.1 i.u.	5 hr.
3	80–90	CHO/fat	200 mg. M.	10 mg. Nic. + 100 mg. glycine	4 days	0.8 i.u. (1st day) 1.0 i.u. (4th day)	12 hr.
4	55–75	CHO/fat	200 mg. M.	10 mg. Nic. + 100 mg. glycine	7 days	0.4 i.u.	12 hr.
5*	60–85	CHO/fat	50 mg. Nic. 200 mg. M. (On last	50 mg. Nic. 50 mg. Nic. day only)	4 days	0.4 i.u.	12 hr.
6	50–80	8% casein 12% gelatin	50 mg. M.	25 mg. glycine	14 days	0.4 i.u. (6th day) 0.4 i.u. (14th day)	12 hr.

* See text.

positive difference in favour of the methionine-fed rats could be observed. The mean difference was 9.9 per cent.

Table IV summarizes the details of the individual experiments. In some experiments a protein-free diet of the following composition was used: 80 per cent dextrose, 16 per cent margarine, and 4 per cent salt mixture; it is referred to as CHO/fat in Table IV. In Exps. 3, 4, and 6 glycine was given to the deficient group of rats in order to provide the same amount of amino-N as the supplemented rats were receiving in the form of methionine. In Exp. 5 nicotinamide was given to both groups for 3 days, and on the last day, during the period of insulin hypoglycaemia, one group received 200 mg. DL-methionine and the other group a further 50 mg. nicotinamide.

The first two experiments were performed in October and November, 1949, and the last four in May and June of this year. In order to produce symptoms of hypoglycaemia in the later experiments it was necessary to use larger doses of insulin than were previously injected.

Excess methionine in normal diet.—Another series of experiments was carried out in which the effect of giving excess methionine to rats eating a normal diet of "rat-cubes" was examined. The details of the diet supplements are summarized in Table V. In comparison with the rats which had been fed on a prepared diet, the duration of the treatment was not so prolonged, and the rats used were of a higher weight. As in the previous series of experiments, the dose of insulin had to be increased as the winter gave place to spring. With the exception of Exps. 7

TABLE V

SUMMARY OF EXPERIMENTS IN WHICH ADDITIONAL METHIONINE WAS GIVEN TO RATS ON NORMAL DIET

M. = DL-methionine (Roche). Nic. = Nicotinamide (B.D.H.)

Exp.	Wt. of rats (g.)	Supplements per rat per day		Duration of experiment	Insulin per 100 g. wt.	Time of death post-insulin
		Methionine group	Control group			
7	85–90	24 mg. M.	10 mg. Nic.	10 days	0.2 i.u.	6 hr.
8	60–110					
9	140–220	200 mg. M.	—	1 day	0.2 i.u.	12 hr.
10	115–160	200 mg. M. (2 days)	—	3 days	0.4 i.u. (1st day) 0.6 i.u. (3rd day)	8 hr.
11–13	130–175	200 mg. M.	—	4 days	0.6 i.u. (2nd day) 0.6 i.u. (4th day)	12 hr.
14	110–160	200 mg. M.	—	4 days	0.8 i.u. (2nd day) 0.8 i.u. (4th day)	12 hr.
15	135–160	200 mg. M.	—	4 days	0.8 i.u. (2nd day) 1.0 i.u. (4th day)	12 hr.

and 8, the control group in this series were fed on the normal "rat-cube" diet, without any other addition.

Table VI gives the percentage of adrenaline found in the adrenal glands of the two groups. The mean difference in Table VI is in the same direction as in Table III, but by themselves the figures in Table VI do not show a significant difference. In Exp. 13 the percentage of adrenaline in the rats given methionine was less than in the controls by 13 per cent. We draw the conclusion that, while there is some indication that the addition of excess methionine may increase the methylating power of the adrenal gland, we cannot define conditions in which this can be demonstrated with regularity.

Experiments with thiouracil.—The rats used in these experiments varied from 100 to 140 g. (mean weight) in different experiments. Each group contained six rats and in each group of any experiment the sex and body-weight distribution were the same. Thiouracil was given in 0.1 per cent (w/v) solution as drinking water. The results are summarized in Tables VII and VIII. Exps. 16 and 17 were carried out at a temperature of 18° to 20° C., the rats being kept indoors. In Exps. 18, 19, and 20 the rat cages were under cover out-of-doors, since the effect of thiouracil is greater when the temperature is lower. The thiouracil produced an increase in size of the thyroid gland. In Exps. 18 and 20 the control rats were given thyroxine.

TABLE VI

RATS HAVING NORMAL DIET GIVEN EXCESS METHIONINE

Type of rat	Adrenaline percentages									
	Exp. 7	8	9	10	11	12	13	14	15	Mean
Control rats ..	67	69	73	59	61	44	78	52	50	61.4
Rats given methionine	82	73	74	60	69	61	67	82	47	68.1

TABLE VII

EFFECT OF THIOURACIL ON ADRENALINE PERCENTAGE

Exp.	Days of thiouracil	Thyroid wt. mg./100 g.		Insulin u./100 g.	Adrenaline percentage		
		Control	Thiouracil		Thiouracil	Control	Thyroxine
16	14	8.5	29.0	0.1	75–100*	75–100*	—
17	12	—	—	—	87	84	—
18	12	8.1	21.0	0.2	83	—	83
19	16	11.0	25.7	0.2	62	63	—
20	25	3.2	18.6	0.8	42	—	30

* The percentage was not precisely determined, but the thiouracil figure was slightly higher than the control.

TABLE VIII

AMOUNTS OF ADRENALINE AND *nor*ADRENALINE IN GLANDS

The figures were obtained by comparing the pressor effect of the extract with that of a mixture of adrenaline and *nor*adrenaline containing the same percentage of adrenaline as the extract

Exp.	Total pressor activity mg./g.		
	Thiouracil	Control	Thyroxine
16	0.68	0.74	—
17	0.7	0.84	
18	0.47	—	0.6
19	0.85	0.60	
20	0.58	—	0.77

In Exp. 18, 50 μg. thyroxine sodium dissolved in 0.01 N-NaOH was given by subcutaneous injection every four days, the total being 0.15 mg. In Exp. 20 the same amount was given at 3-day intervals during 25 days, the total being 0.4 mg. In Exp. 16 the adrenaline percentage in the two groups of rats was not accurately determined, though it lay between 75 and 100 per cent. A direct comparison of the two extracts showed that the percentage was slightly higher in the rats given thiouracil. In Exp. 17 no insulin was injected. In Exps. 17, 18, and 19 there was no difference in the percentage of adrenaline in the control and thiouracil groups, while in Exp. 20 it was very low in both groups, though actually higher in the thiouracil group than in the thyroxine group.

The total amine content measured by the pressor activity was, in all experiments except Exp. 19, lower in the thiouracil rats than in the control rats. In both experiments in which thyroxine was given it was higher in the thyroxine group. The figures are given in Table VIII.

Discussion

We have been able to demonstrate that in rats with some degree of methionine deficiency the administration of methionine increases the rate of methylation of *nor*adrenaline. However, although the effect was seen in each experiment, it was not a large effect. Probably the actual number of methyl groups required to methylate *nor*adrenaline in the adrenal medulla is small in relation to the number in the body.

In rats in which no attempt was made to create a methionine deficiency, the giving of excess methionine did not raise the percentage of adrenaline in the adrenal medulla to an extent which was significant, though there was a change in this direction. The result does not suggest that the proportion of adrenaline in the adrenal medulla of human subjects can be raised by feeding methionine, and so it becomes unlikely that methionine can benefit asthmatics by providing them with more adrenaline in the adrenal medulla. If some individuals have difficulty in methylating *nor*adrenaline in the adrenal medulla, the limiting factor seems not to be the supply of methyl groups.

The failure to observe retarded methylation in rats given thiouracil in order to diminish thyroid activity was unexpected. In each of the experiments the percentage of adrenaline in the glands of rats treated with thiouracil was just as great as in control rats, or even in rats given thyroxine. It is possible that a longer period of thiouracil administration would have produced a different result, though the enlargement of the thyroid gland showed that a diminution in circulating thyroxine must have occurred in each experiment. The negative result at least serves to emphasize the positive result with methionine.

SUMMARY

1. If rats are given a diet deficient in methionine and choline, and after a period of 2 weeks are injected with sufficient insulin to produce symptoms of hypoglycaemia, the percentage of adrenaline in the adrenal medulla when the rats are killed 4–12 hr. later is less than it is in rats given the same diet supplemented with methionine. Whereas the mean percentage of adrenaline was 70.6 in deficient rats, it was 80.5 in rats given the same diet supplemented by methionine. A difference in the same direction was observed in each of six experiments.

2. When a similar comparison was made between rats given a normal diet and others given methionine in addition, the mean results did not differ significantly, though the mean percentage of adrenaline was again higher in the rats receiving methionine.

3. The administration of thiouracil (in 0.1 per cent solution as drinking water) to rats for periods up to 25 days does not affect the percentage of adrenaline in the adrenal medulla when the rats are killed 12 hr. after injection with insulin, and therefore does not appear to diminish the rate of methylation of *nor*adrenaline.

The authors take great pleasure in expressing their thanks to Professor J. H. Burn for his advice and help throughout this investigation. This work was done by one of us (D. E. H.) during the tenure of a Fellowship awarded by the National Research Council of Canada, and by the other of us (R. H. O. P.) during the tenure of a grant from the Medical Research Council.

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