SUMMARY

1. The chromatographic separation of steroid 3:5-dinitrobenzoates on alumina and silica has been studied.

2. The non-ketonic alcohols from the urines of normal men and women and three cases of adrenal dysfunction have been esterified and partitioned on alumina.

3. The excretion patterns obtained from the urine of patients with adrenal tumour, adrenal hyperplasia and Cushing's syndrome differed from normal and from one another, and these differences may be of diagnostic value.

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The Inheritance in the Rat of the Capacity to Eliminate Nicotinamide Methochloride

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In earlier investigations by one of us on the metabolism of nicotinamide in rats, it had been observed that the two strains of rats used showed great differences in their daily elimination of nicotinamide methochloride and in their response, i.e. the increase of this elimination, to extra-dietary administration of nicotinamide (Ellinger & Abdel Kader, 1949). Both these factors are remarkably stable for the same individual on different days under standard conditions of feeding and housing. The methylation of nicotinamide occurs in the liver (Perlzweig, Bernheim & Bernheim, 1943; Ellinger, 1948) and is catalysed by an enzyme system studied in greater detail and called nicotinamide methyl kinase by Cantoni (1951).

The original experiments, already published as a preliminary communication (Ellinger, 1949), have

been extended in various directions and the results analysed statistically. It is the object of this paper to examine in detail the inheritance of the nicotinamide-methylating mechanism.

EXPERIMENTAL

Plan of experiments

The strains of rats used were, as in earlier experiments, a black hooded strain bred in the Lister Institute for more than 25 years, and an albino strain bred by one of the authors (P. E.) for about 10 years, both inbred for more than thirty generations. The coat colour genes which differentiate these two strains are discussed in more detail below.

In a previous report on twenty-one adult rats of each strain (Ellinger & Abdel Kader, 1949) the average daily nicotinamide methochloride output of the hooded strain had been 960 μg . (range, 450–1700 μg .), and that of the albino

strain 100 μg . (30–260 μg .). The average response to 10 mg. of extra-dietary nicotinamide had been 5780 μg . (1890–8130 μg .) for the hooded, and 1350 μg . (630–4120 μg .) for the albino strain. In the daily nicotinamide methochloride output there was thus a distinct gap between the two strains. The average responses also differed considerably, but the two series of responses overlapped.

In the present experiments, output and response have been determined for a number of rats of the two inbred strains, and also for the progeny of a number of generations of cross-breeding. The experiments were carried out over two distinct periods, 1948–9 and 1951.

In 1948–9, four litters of F_1 offspring were produced by crossing the two strains. By mating the F_1 rats *inter se* seven litters of F_2 offspring were obtained. Random mating within the F_2 and succeeding generations failed for various reasons; quite a number of matings proved sterile. (Matings were considered to be sterile when no offspring were born after 3 months.) Five matings of F_2 rats each produced two litters of F_3 offspring.

In 1951 four litters of F_1 offspring were again obtained. Individuals of the F_1 generation were then crossed back to the two inbred strains. Twenty litters were obtained from the $F_1 \times$ albino backcross, and twenty-four from the $F_1 \times$ hooded backcross.

It was found useful for descriptive purposes to classify rats, from their average daily nicotinamide methochloride output, into three grades: (1) low, less than 150 μg .; (2) medium, between 150 and 450 μg .; and (3) high, 450 μg . or more. Responses were also divided into three grades: (1) low, less than 1500 μg .; (2) medium, between 1500 and 4000 μg .; and (3) high, 4000 μg . or more.

Methods

The rats were kept on the mixed diet described by Ellinger & Abdel Kader (1949). To collect the urines the rats were kept for 3 or 4 days in metabolism cages at a room temperature of 20–25°. For the estimation of the average daily nicotinamide methochloride output, 24 hr. urine samples were collected for 2 or 3 consecutive days. When the single values found for the daily output differed from each other by more than 10%, collection of urine was continued until the values were stable. Then, in order to determine the response to extra-dietary nicotinamide, the rats were given on the following day 1 ml. 1% (w/v) aqueous nicotinamide, intraperitoneally. In the urine passed during the 24 hr. following the injection, the nicotinamide methochloride elimination was estimated and the response was calculated by deducting the average daily nicotinamide methochloride output on the standard diet from that after injection.

These experiments were carried out when the rats were 8–16 weeks old and weighed between 120 and 350 g. It had been found in preliminary experiments that the individual nicotinamide methochloride output and response were very nearly constant and did not vary markedly between the ages of 4 and 20 weeks if the diet was constant; they were little influenced by weight. Before the urines were collected and tested the animals had been kept on a similar diet and under similar conditions of housing and temperature. Some differences in urinary output might be due to the habit of some rats of storing large amounts of urine in the bladder and emptying the bladder only at long irregular intervals.

The nicotinamide methochloride estimation was carried out by the slightly modified acetone method of Huff & Perlzweig (1947).

RESULTS

Breeding

Both inbred strains were good breeders, having less than 10% sterile matings. The size of the litters varied with the season, the extremes being three and sixteen. The cross-breeding of the two strains in the first period was much less successful; of six matings of albino males with black hooded females, three succeeded with litters of five or six, one mating produced only one offspring and the other two were sterile. Six matings of black hooded males with albino females were all sterile.

From matings of the succeeding generations it was often difficult to obtain offspring. The most successful matings were those of rats with similar outputs. Matings of low output males with high output females were fairly successful, but offspring from the others, particularly from high output males with low or medium output females, were hardly obtainable, and only after a large number of matings had been tried. Strictly random matings amongst the F_2 generation, and for succeeding generations, were therefore not possible. During the second period the cross-breeding was a little more successful and those types of mating which had been sterile in the first period were more successful during the second; but even here a high percentage of the attempted matings were failures.

The back-crossing between the F_1 generation and the parent strains was much less difficult.

Coat colour

In the first period, 1948–9, all the F_1 generation were agouti hooded. The F_2 generation showed segregation into black hooded, agouti hooded and albino. In this period the rats used from the albino strain were homozygous for dominant agouti, recessive albino and recessive hooded ($AAcchh$). The black hooded rats were homozygous for recessive non-agouti, dominant coloured, and recessive hooded ($aaCChh$). All the coloured descendants were hooded, since no segregation of this gene took place. Rats of the first cross (F_1) were agouti hooded ($AaCchh$). In the F_2 generation there were sixteen agouti hooded, ten black hooded and thirteen albino; these frequencies do not differ significantly from the expected 9:3:4 ratios (the value of χ^2 being 3.68 on 2 degrees of freedom, corresponding to a probability between 0.1 and 0.2).

In the second period, 1951, all the F_1 generation were black hooded. About half the offspring of the $F_1 \times$ albino backcross were black hooded, and half albino. The offspring of the $F_1 \times$ hooded backcross

were all black hooded. In this period four different albino parents were used, and it appears that the albino strain was now carrying the recessive non-agouti gene. The only gene now segregating was that for coloured versus albino. Denoting the genotypes of the albino and hooded strains by *cc* and *CC*, respectively, the F_1 rats were *Cc*; the backcrosses with the albino strain were *Cc* and *cc*—about half black hooded and half albino phenotypes; the backcrosses with the black hooded strain were *Cc* and *CC*—all black hooded phenotypes.

Inheritance of nicotinamide methochloride output

Table 1 shows the distributions of the logarithm (to base 10) of the output in rats from the two inbred strains, from their first cross (F_1), from the backcross of F_1 with each inbred strain, from the progeny of crosses within the F_1 generation (F_2), and from F_3 litters with parents taken from the F_2 generation. The results for the parent strains, and for the F_1 generation in the two periods are very similar, and both periods are combined in Table 1. The results are shown in the form of histograms in Fig. 1*a*; the frequencies are here expressed as percentages, the grouping interval being twice that shown in Table 1. Table 1 and Fig. 1 show that the distributions for the two parent strains are well separated, whilst that for the F_1 generation occupies an intermediate position with very little overlapping. In fact, according to the classification adopted above, the albino, black hooded and F_1 groups of rats are

almost entirely low, high and medium eliminators, respectively. The intermediate position of the F_1 generation strongly suggests that output is inherited multifactorially (i.e. controlled by several genes).

As would be expected for an inherited characteristic, log. output shows more variation in the two backcross distributions than in either of the parent strains or the F_1 , and still more in the F_2 generation.

In the study of the genetics of continuous variation care must be taken to choose a suitable scale of measurement. Mather (1949) has emphasized the importance of two considerations: (a) if the inheritance is multifactorial, i.e. controlled by more than one gene, the scale should be chosen so that the effects of the different genes are additive; (b) the environmental variation, to which even genetically identical individuals are subject, should as far as possible be of constant magnitude at all points of the scale. Two consequences of (a) are that the mean values in successive generations (obtained by random mating) should approach the mid-point between the means for the two inbred strains, and that the mean for each of the first backcrosses should be mid-way between those for the corresponding inbred strains and the F_1 .

The mean values in Table 1 show that the logarithmic scale is satisfactory as far as criterion (a) is concerned. The parent means are 1.899 and 2.928. The F_1 and F_2 means (2.412 and 2.449) are both very close to the mid-point, 2.414. (The F_3 mean is

Table 1. *Distributions of log. output of nicotinamide methochloride*

(The symbols M. and F. indicate, respectively, the values of log. output for the male and female F_2 parents of the F_3 litters.)

Log. output (μ g.)	Number of rats						Number of rats. F_3 litters. (Each of the groups A-E consists of two litters from a single pair of F_2 parents)				
	Albino	Hooded	F_1	$F_1 \times$ albino	$F_1 \times$ hooded	F_2	A	B	C	D	E
< 1.50	2	—	—	—	—	1	—	—	—	—	—
1.50–	7	—	—	7	—	—	—	—	—	—	—
1.65–	9	—	—	2	—	1	—	—	2	—	3
1.80–	6	—	—	5	—	1	—	1	2	3	5 F.
1.95–	17	—	—	18	1	5	—	F.	—	2	3 M.
2.10–	9	—	5	34	6	6	—	—	4 M.	3 M.	2
2.25–	—	—	19	47	24	6	—	2	2 F.	2	4
2.40–	—	—	17	17	29	4	—	3	4	2	—
2.55–	—	1	10	10	25	2	—	M.	6	—	—
2.70–	—	17	—	1	39	5	2	1	2	3 F.	1
2.85–	—	23	—	1	43	3	2	—	—	1	—
3.00–	—	12	—	—	31	3	9 F.	—	—	6	—
3.15–	—	4	—	1	4	2	4 M.	—	—	—	—
3.30–	—	—	—	—	—	—	1	—	—	—	—
Total no. of rats	50	57	51	143	202	39	18	7	22	22	18
No. of litters	7	7	8	20	24	7	2	2	2	2	2
Variance	0.0474	0.0176	0.0178	0.0675	0.0708	0.1732	—	—	—	—	—
Mean	1.899	2.928	2.412	2.243	2.719	2.449	—	—	—	—	—
S.E.M.	0.031	0.018	0.019	0.022	0.019	0.067	—	—	—	—	—
Variance about litter means	0.0337	0.0135	0.0109	0.0453	0.0636	0.1761	—	—	—	—	—

irrelevant here, since this generation was not bred by random mating of the F_2 's.) The backcross means, 2.243 and 2.719, are close to the expected values of 2.156 and 2.670. The logarithmic scale has not been quite as effective in equalizing 'environmental' variance. The inbred strains are probably not com-

satisfy both (a) and (b), and for the present purpose the logarithmic scale is quite adequate.

The backcrosses are particularly informative in indicating the multifactorial nature of the inheritance. If output were controlled by one gene alone with incomplete dominance half the rats in each backcross would be homozygous for this gene (as were the corresponding inbred rats), and half would be heterozygous like the F_1 . We should therefore expect a bimodal distribution in each backcross, the two modes in each case being separated by a distance of about 0.4 logarithmic unit. If, on the other hand, the inheritance were multifactorial, the backcross distributions would be unimodal. Table 1 shows no suggestion of marked bimodality for the albino $\times F_1$ backcross, but there is a slight ambiguity about the hooded $\times F_1$ backcross. Now, the true nature of these backcross

Fig. 1. Percentage frequency distributions of log. output and log. response in inbred and crossed strains. Means are indicated by arrows. For each F_3 group, the corresponding value for each F_2 parent is indicated below the histogram. (a) Log. output; (b) log. response.

pletely homozygous, in view of the change in the albino stock between 1948 and 1951, and part of the variation described here as 'environmental' may be genetical. It will be convenient to use the term 'environmental' to include both truly environmental variation and the residual genetical variation. The 'environmental' variance is appreciably greater in the albino strain than in the hooded or F_1 , perhaps because the residual genetical component is greater in the albino than in the hooded strain. It seems unlikely that any simple scale will completely

Table 2. Distributions of deviations of log. output from litter means (m), in the backcrosses

Log. output	$F_1 \times$ albino	$F_1 \times$ hooded
(m - 0.825) -	—	—
(m - 0.675) -	1	—
(m - 0.525) -	5	11
(m - 0.375) -	9	32
(m - 0.225) -	38	30
(m - 0.075) to (m + 0.075)	33	47
(m + 0.075) -	45	40
(m + 0.225) -	10	35
(m + 0.375) -	1	6
(m + 0.525) -	—	1
(m + 0.675) -	1	—
No. of rats	143	202

distributions is partly obscured by a small component of environmental variance between litters (as may be seen by comparing the total variance of each distribution with the variance within litters, as shown in Table 1). In fact, the means for the albino $\times F_1$ litters showed a trend of about -0.3 logarithmic unit over the period of 4 months during which these results were obtained. The hooded $\times F_1$ backcross results showed some heterogeneity between litters, but no consistent trend. In order to clarify this point, each backcross result has been expressed as a deviation from its litter mean, and the distributions formed by these deviations are shown in Table 2. The $F_1 \times$ hooded backcross clearly forms a unimodal distribution. The $F_1 \times$ albino results are a little ambiguous, however, but the distance between the two apparent modes is less than would be expected on the one-gene theory, and the apparent bimodality may easily be due to sampling fluctuations.

The fact that the F_1 mean is very close to the midpoint between the means for the two parents, shows that (on this scale) there is no consistent dominance in either direction.

Table 3. *Distributions of log. response*

(The symbols M. and F. indicate, respectively, the values of log. response for the male and female F_2 parents of the F_3 litters.)

Log. response (μg . nicotinamide methochloride)	Number of rats				Number of rats. F_3 litters. (Each of groups A-E consists of two litters from a single pair of F_2 parents)				
	Albino	Hooded	F_1	F_2	A	B	C	D	E
< 2.50	—	—	—	1	—	—	—	M.	—
2.50–	2	—	—	1	—	—	—	—	—
2.65–	1	—	—	—	—	—	—	—	—
2.80–	8	—	—	1	—	—	—	—	4 F.
2.95–	1	—	1	1	—	—	2 M.	—	8
3.10–	—	—	9	3	—	—	4	F.	1
3.25–	—	—	17	4	—	1	5	4	4
3.40–	—	3	11	8	F.	3	3 F.	7	M.
3.55–	—	9	1	2	6	3	5	5	1
3.70–	—	—	—	3	9 M.	M.	3	3	—
3.85–	—	—	—	1	2	F.	—	2	—
4.00–	—	—	—	—	1	—	—	1	—
Total no. of rats	12	12	39	25	18	7	22	22	18
No. of litters	3	2	5	4	—	—	—	—	—
Variance	0.0169	0.0032	0.0154	0.1211	—	—	—	—	—
Mean	2.837	3.575	3.330	3.372	—	—	—	—	—
S.E.M.	0.037	0.016	0.017	0.070	—	—	—	—	—
Variance about litter means	0.0183	0.0026	0.0127	0.1201	—	—	—	—	—

Inheritance of response

Table 3 shows the distribution of the logarithm of the response in rats from the two inbred strains, and from the F_1 , F_2 and F_3 generations. The results are presented in the form of histograms in Fig. 1*b*. The number of observations available is smaller than for output; in particular, no responses were measured for the backcrosses. The results are consistent with the hypothesis of multifactorial inheritance with a tendency to dominance in the direction of high response (the F_1 mean being nearer to the hooded mean than to the albino mean), but the distinction between this hypothesis and that of a single gene is not as clear as in the case of output. A rather anomalous feature is the extremely low response found in the F_2 father of the *D* group of F_3 offspring, a response apparently unrelated to those of the offspring.

Relationship between output and response

It is of some interest to determine whether response is genetically determined by the output, whether the two measurements are genetically independent or whether there is some incomplete genetical correlation between the two.

For each of the 175 rats, the distributions of whose responses are shown in Table 3, a corresponding output is available. Figs. 2–4 show, respectively, the scatter diagrams of log. output and log. response for the inbred and F_1 rats, for the F_2 generation, and for the five groups of F_3 rats (each group

consisting of two litters from the same parents). Table 4 summarizes the essential features of these diagrams, i.e. the regression coefficient of log. response on log. output, its standard error and the residual variance about the regression line.

The results for the inbred strains and for the F_1 offspring show the relationship between the purely 'environmental' components of output and response. It is clear from Fig. 2 and from the non-significant regression coefficients in Table 4 that these components are at most only slightly correlated, perhaps a rather surprising result.

Fig. 3 and Table 4 show that for the F_2 generation the regression of log. response on log. output is positive, but not significant. The important point here, though, is that the residual variation is very much greater than the purely 'environmental' components discussed above. This means that, for a given value of output, the response is still subject to considerable segregation. Response is not completely genetically determined by output.

In two of the five F_3 groups illustrated in Fig. 4, the regression is positive and significant. There is apparently some genetical relationship between the two measurements, which would presumably have manifested itself in a significant regression in the F_2 results, had they been numerous enough.

The results summarized in this and the two preceding sections, then, suggest that both output and response are genetically controlled by a number of genes. The two sets of genes have some, but not all, members in common.

Table 4. Regression coefficient of log. response on log. output, with residual variance about the regression line

	No. of rats	Regression coeff. $\pm$ standard error	Significance	Residual variance and degrees of freedom
Albino	12	0.315 ± 0.170	—	0.0133 (10 d.f.)
Hooded	12	-0.056 ± 0.239	—	0.0037 (10 d.f.)
F_1	39	-0.037 ± 0.134	—	0.0140 (37 d.f.)
F_2	25	0.321 ± 0.181	—	0.1111 (23 d.f.)
F_3 { A	18	0.240 ± 0.166	—	0.0117 (16 d.f.)
B	7	0.189 ± 0.105	—	0.0051 (5 d.f.)
C	22	0.551 ± 0.103	($P < 0.001$)	0.0237 (20 d.f.)
D	22	0.370 ± 0.057	($P < 0.001$)	0.0127 (20 d.f.)
E	18	0.027 ± 0.193	—	0.0451 (16 d.f.)

Fig. 2. Relationship between log. response and log. output in albino, black hooded, and F_1 rats. $\square$, Albino; $\times$, F_1 ; $\bullet$, black hooded.

Fig. 4. Relationship between log. response and log. output in five groups of F_3 rats. $\circ$, Group A; Δ , group B; $\bullet$, group C; $\times$, group D; $\square$, group E.

Sex differences in output and response

In view of the component of environmental variation between litters, it seemed desirable to examine the possible effects of sex within litters. Since the sex ratio varies from litter to litter, the method of unweighted means (Kendall, 1951) has been used.

Table 5. Mean differences in log. output between males (M.) and females (F.), within litters

	M. - F. difference $\pm$ standard error	Degrees of freedom for standard error
Albino	-0.005 ± 0.072	36
Hooded	-0.010 ± 0.041	40
F_1 { Albino M. parent	0.021 ± 0.047	32
Albino F. parent	-0.071 ± 0.037	

In Table 5 the estimated mean differences in log. output between the sexes are given, with their standard errors, for the two inbred strains and for the F_1 offspring of the two types of first cross. (One

Fig. 3. Relationship between log. response and log. output in F_2 rats.

litter of the hooded strain, and two of the F_1 , were all of one sex and are therefore not used in this comparison.)

The sex differences are in no instance significant. In the reciprocal F_1 crosses they are of opposite sign, but their difference, 0.092, has a standard error of 0.060, and is thus not significant. The data therefore provide no evidence either of a sex difference in output, or that sex-linked genes play an important part in the inheritance of output.

Table 6. *Mean differences in log. response between males (M.) and females (F.), within litters*

	M. - F. difference ± standard error	Degrees of freedom for standard error
Albino	0.015 ± 0.109	6
Hooded	-0.004 ± 0.039	8
F_1 { Albino M. parent Albino F. parent	0.125 ± 0.085 0.047 ± 0.038	25

The corresponding sex differences in log. response are shown in Table 6. As with output, there is no evidence of a sex difference or sex linkage in response.

Relationship of output and response to coat colour

Table 7 shows the mean values, with their standard errors, of log. output in the F_2 and F_3 generations, and in the $F_1 \times$ albino backcross, grouped according to the three coat colour pheno-

types. Only groups *A* and *E* of the F_3 generation are shown; groups *B*, *C* and *D* were, respectively, all agouti hooded, all black hooded, and all albino. Table 8 shows the relationship of log. response to the coat colour phenotypes. (No responses were obtained in the backcrosses.)

The method of unweighted means, which was used for sex differences, is not convenient here since in many litters not all the phenotypes are represented. Differences between litters have therefore been ignored.

The only significant differences between phenotypes in Tables 7 and 8 occur in the F_2 generation. Analyses of variance show that the differences in log. output between the phenotypes are significant at the 0.1% level, whilst the differences in log. response are significant at the 5% level. For both log. output and log. response the agouti hooded rats show the highest mean values, and the black hooded ones the lowest.

These differences are clearly not due to pleiotropic action of the genes determining coat colour, for the relationship occurs only in the F_2 generation. Furthermore, the results in the 1951 experiments, when the albino strain carried the non-agouti instead of the agouti allele, fitted in extremely well with the previous results. The explanation presumably is that one of the genes determining output and one of those determining response are linked with the agouti gene. The effect may even be due to one gene which affects both output and response.

Table 7. *Relationship of log. output to coat colour*

	Phenotypes					
	Agouti hooded		Black hooded		Albino	
	No. of rats	Mean ± S.E.	No. of rats	Mean ± S.E.	No. of rats	Mean ± S.E.
(a) 1948 experiments						
F_2	16	2.701 ± 0.082	10	2.035 ± 0.103	13	2.452 ± 0.091
F_3 { <i>A</i>	14	3.106 ± 0.039	—	—	4	2.940 ± 0.072
<i>E</i>	4	1.952 ± 0.135	8	2.151 ± 0.095	6	2.032 ± 0.110
(b) 1951 experiments						
$F_1 \times$ albino backcross	—	—	70	2.252 ± 0.031	73	2.235 ± 0.030

Table 8. *Relationship of log. response to coat colour*

(1948 Experiments)

	Phenotypes					
	Agouti hooded		Black hooded		Albino	
	No. of rats	Mean ± S.E.	No. of rats	Mean ± S.E.	No. of rats	Mean ± S.E.
F_2	9	3.633 ± 0.099	6	3.218 ± 0.121	10	3.229 ± 0.094
F_3 { <i>A</i>	14	3.736 ± 0.031	—	—	4	3.745 ± 0.057
<i>E</i>	4	3.113 ± 0.107	8	3.154 ± 0.075	6	3.047 ± 0.087

The F_2 group *E* had an albino father and a black mother. The distribution of coat colour phenotypes in this group (as given in Table 7) shows that the albino parent was heterozygous for the agouti gene. If, as seems likely, a gene making for high output is associated with the agouti gene, the non-significance of the difference in mean log. output between the agouti and black progeny in group *E* may well be due to the albino parent being a recombinant, and thus lacking the hypothetical gene. Indeed, the fact that this albino F_2 had an output below any of the other albino or agouti F_2 rats strengthens the hypothesis that it was a recombinant.

The F_2 results show that, for the gene or genes responsible for the association of output and response with coat colour phenotypes, the allele making for high output or response was carried by the albino strain, and that making for low output or response was carried by the hooded strain. This contrast between the two inbred strains is in the opposite direction to their overall difference, for the albino strain had the lower output and response. The effect of the gene or genes linked with agouti is therefore overridden by that of other genes which act in the opposite direction.

DISCUSSION

The differences in nicotinamide methochloride output and response by the two strains used could have been caused by differences in nicotinamide intake from food and from release by the intestinal flora, differences in the available methyl donors, differences in the elimination mechanism of the kidney for nicotinamide methochloride or differences in the efficiency of the nicotinamide-methylating mechanism (Ellinger & Coulson, 1944); this mechanism is located in the liver (Perlzweig *et al.* 1943; Ellinger, 1946, 1948; Cantoni, 1951). It could also be caused by differences in the possible oxidation of nicotinamide methochloride to 1-methyl-6-pyridone-3-carbonamide (Knox & Grossman, 1946; Holman & De Lange, 1949) or in the possible formation of some unknown products such as 'pseudo-nicotinamide methochloride' found in the urine of guinea pigs after injection of nicotinamide (Ellinger & Abdel Kader, 1949). The first possible cause could be eliminated by keeping the animals under identical conditions of feeding and housing. Methyl donors were plentiful and administration of either choline or methionine did not increase the nicotinamide methochloride elimination, or response to injected nicotinamide (Ellinger & Abdel Kader, 1949). The kidney elimination could not be involved since intraperitoneally injected nicotinamide was eliminated by both strains to the same degree, i.e. 60-70% (Ellinger & Abdel Kader, 1949). Oxidation to the pyridone could be

excluded since this product was not found in the urine of rats (Perlzweig, Rosen & Pearson, 1950; Ellinger, 1950). Thus the cause for the differences in the nicotinamide methochloride output and response to the injected nicotinamide must have been due either to the difference in the efficiency of the nicotinamide-methylating mechanism of the liver, or to differences in the possible formation of unknown metabolites.

The control of enzymic processes in mammals by heredity has been described for the following phenomena: the presence or absence of atropine esterase in the rabbit, the presence or absence of xanthophyllase in rabbits and the quantity of uricase present in the serum of Dalmatian dogs. It was first shown by Fleischmann (1910) that certain rabbits can destroy atropine while others cannot; this renders some rabbits immune towards atropine contained in the fodder plants. This phenomenon has been confirmed by numerous investigators, and in a genetical study Sawin & Glick (1943) have shown that the presence of the atropine-destroying enzyme is controlled by a single gene borne in the same chromosome as the gene for the extension of the black pigment in the coat. The gene was described as incompletely dominant. The enzyme is not present at birth, but appears at about the age of 1 month, and is said to have a higher concentration in females than males. There were no intermediate offspring with reduced atropine esterase content. The offspring were either positive or negative.

If xanthophyllase, which destroys carotene or xanthophyll in normal rabbits, is absent from the rabbit liver the fat of the rabbit is yellow. The genetics of the yellow fat character was studied by Pease (1928), who found that this condition is due to a single recessive factor, genetically linked with albinism and borne in the same chromosome as a colour gene. Castle (1933) confirmed Pease's results and showed that the yellow fat is also linked with brown hair and skin pigmentation.

While these two enzymes are either absent or present in the crossbreds, the third inherited enzymic function studied so far, the action of uricase in the dog, i.e. the elimination of purine derivatives either as allantoin or as uric acid by the dog is a question of quantity. Man and ape eliminate purine derivatives mainly as uric acid, while all other mammals eliminate them as allantoin. Benedict (1916) first observed that the spotted Dalmatian dog behaves in this respect like men and apes; unlike other dogs, they eliminate mainly uric acid. By cross-breeding these Dalmatian dogs with ordinary dogs, Onslow (1923*a, b*) made it probable that the high uric acid elimination by the Dalmatian is controlled by an inherited factor which is in some way linked with the factors controlling the pigment distribution. Onslow's experiments were repeated

by Trimble & Keeler (1938). By cross-breeding Dalmatian dogs with ordinary dogs, they produced offspring with exclusively low uric acid elimination in the F_1 generation. By cross-breeding the offspring *inter se* they obtained an F_2 generation with either low or high uric acid elimination, but there was no offspring with intermediate uric acid elimination; no sex linkage was observed. By cross-breeding the F_1 generation *inter se*, or by backcrossing the F_1 generation with the parent Dalmatian generation, it was observed that the high uric acid elimination was dependent on the presence of probably one, but not more than two, recessive genes. There was no evidence of linkage with the gene, or genes, controlling the pigment distribution of the coat. According to Friedeman & Byers (1948) the fundamental anomaly in the Dalmatian is its inability to reabsorb uric acid from the glomerular filtrate.

From all these genetical findings the inheritance of the nicotinamide-methylating enzyme of the rat's liver differs in that by the mating of the low excretors with the high ones an intermediate type results, which segregates in the following generation. Whereas in the original inbred strains the distributions of the amount of nicotinamide methochloride elimination in the parent and first cross generations were fairly well separated, the distributions of response overlapped considerably. These observations suggested that the genetical basis of the response was probably not so clear as that of the daily nicotinamide methochloride elimination. The statistical analysis has confirmed this suggestion.

SUMMARY

1. Two strains of pure-bred rats, one albino and one black hooded, with low and high nicotinamide methochloride elimination and response to extra-dietary nicotinamide, respectively, were cross-bred in order to test whether the amount of nicotinamide methochloride elimination and the response were genetically controlled. For this purpose nicotinamide methochloride output and response of the offspring of several generations of the cross-breds and back-crossings were examined, and related to coat colour.

2. Output and response are inherited multifactorially. The two sets of genes controlling the two measurements have some, but not all, members in common. The data provide no evidence of a direct effect of sex, or that sex-linked genes play an important part in the heredity of output or response. One of the genes controlling output and one of those controlling response (possibly the same gene) are linked with the agouti gene.

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