

## **Aversion–preference patterns in amino acid- or protein-deficient rats: a comparison with previously reported responses to thiamin-deficient diets**

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The purpose of the present experiments was to extend previous data on the strategy used by adult rats to select feed appropriately when faced with diets devoid of protein or an essential amino acid (EAA), and to compare this strategy with that used when facing vitamin (thiamin) deficiency. Rats fed on either balanced or deficient (EAA or protein) diets were offered a choice between a novel deficient and a familiar (deficient or corrected) diet and their choice was monitored. It was shown that protein- and EAA-deficient rats acquired an aversion for their corresponding familiar devoid diet, which by itself promoted a neophilia for the novel diet. This neophilia was not non-specific because protein-deficient rats facing a choice between a protein-devoid and an EAA-devoid diet did exhibit neophilia but only in the short term (less than 5 h), and then switched to a preference for the familiar protein-devoid diet. These results show that, in contrast to the case of vitamin deficiency, the protein- or EAA-devoid diet-induced neophilia can be rapidly reversed if the novel feed happens to be more deleterious than the familiar, inappropriate one. This behaviour suggests the existence of sensitive mechanisms able to reveal within a short time the EAA inadequacy of the ingested feed and to adapt the choice for the most appropriate feeds more promptly than in the face of thiamin deficiency. Thus it appears that balancing EAA is more urgent than balancing thiamin.

**Dietary choice: Nutrient-preference: Conditioned taste aversion: Thiamin: Specific appetites**

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Specific hungers for dietary micronutrients have been reported for many years (Harris *et al.* 1933; Richter, 1956). The only well-established innate appetite is for Na, which possesses its own gustatory detector; most of the other micronutrient appetites are learned (Rozin, 1976; Booth 1985). Since the studies of Harris *et al.* (1933), it has been known that vitamin B<sub>1</sub> (thiamin)-deficient rats have a preference for feeds containing the needed nutrient. Scott & Verney (1947) suggested that the preference for thiamin-containing diets exhibited by thiamin-deficient rats is a learned phenomenon. Rozin *et al.* (1964) and Rozin (1965) demonstrated that specific thiamin appetite is more an expression of an acquired aversion for the thiamin-devoid diet than a preference for the novel diet. Rats that have experienced a thiamin-devoid diet become more neophobic than normal rats (Rozin, 1968).

However, when given a choice between a novel deficient diet and a familiar diet that was deficient and therefore aversive, but is now corrected, the rats choose the former. That is, rats prefer any novel diet, deficient or not, rather than a former familiar deficient diet, which has become aversive, even if this diet is now corrected. This phenomenon was

designated a 'non-specific neophilia' (Rodgers, 1967). Zahorik & Maier (1974) demonstrated that rats showed a preference for feeds associated with recovery from thiamin deficiency over familiar safe feeds, but an aversion seems to be easier to learn than a preference (Rozin, 1976). Booth (1985) criticized this concept, arguing that the models studied by Rozin and Garcia, i.e. aversion induced by severe nutritional deficiency or by toxic events, are not so common in nature and that learned sensory preferences induced by a mild nutritional deficiency are more frequently encountered.

Specific hungers for essential amino acids have also been demonstrated (Halstead & Gallagher, 1962; Rogers & Harper, 1970). Amino acid-deficient rats acquire an aversion for amino acid-deficient diets (Simson & Booth, 1974*a, b*) and a preference for diets well-balanced in amino acids (Gietzen *et al.* 1992; Naito-Hoopes *et al.* 1993). As in the case of thiamin, preferences are more difficult to acquire than aversion. After a neurobiochemical phase of rapid recognition of the deficiency, the animal develops a subsequent conditioned taste aversion for the sensory characteristics that allow discrimination of the amino acid-deficient feed (reviewed by Gietzen, 1993).

The dietary self-selection technique has been used widely to demonstrate the existence of specific hungers in deficient animals and the regulation of nutrient intake in normal animals. Using such a technique, Leung *et al.* (1968) demonstrated that normal rats prefer a protein-free diet, which does not support growth, rather than an amino acid-imbalanced diet. However, when normal rats have a choice between an amino acid-imbalanced diet and a corrected diet, they successfully choose the well-balanced diet.

The present studies were designed to compare the mechanisms underlying specific amino acid appetites with those involved in the case of thiamin. In order to make the comparison with thiamin appetite easier, the types of choice protocols in our experiments were closely modelled on those used with thiamin by Rozin and Rodgers (Rozin *et al.* 1964; Rozin, 1965; Rozin & Rodgers, 1966; Rodgers, 1967). The first issue was to determine the respective roles of induced aversion and non-specific neophilia in the ongoing choices made by amino acid-deficient rats or by normal rats. For that purpose the daily pattern of the choice between two versions of a diet, one of them being amino acid-deficient, was characterized in normal and in amino acid-deficient rats (Expt 1). In order to evaluate the role of non-specific neophilia the daily pattern of the choice between a protein-free diet and a familiar amino acid-devoid but now corrected diet was characterized in amino acid-deficient rats (Expt 2). In order to clarify further the importance of the non-specific neophilia the daily pattern of the choice between a protein-free diet and an amino acid-devoid diet was assessed in protein-deficient rats (Expt 3). The second question was whether there is an innate amino acid preference. Rodgers (1967) stated that an innate nutrient appetite would be indicated if nutrient-deficient rats showed an immediate preference for the previously devoid but now corrected diet over any novel deficient diet. To address this question the short-term choice pattern between a novel protein-free diet and a familiar amino acid devoid but now corrected diet was characterized in amino acid-deficient rats (Expt 2b).

## MATERIALS AND METHODS

### *Animals*

The subjects were adult male Wistar rats (Iffa-Credo). They were individually housed in stainless steel wire cages at 22 (SEM 2)°, on a 12 h light-dark cycle (06.00–18.00 hours). In Expts 1a, 2b, 2c, and 3, rats had been exposed to one of the diets. In Expts 1b, 1c and 2a,

rats were naïve to the diets and the choice paradigms. All experimental procedures conformed to the guidelines of the National French Animal Care Committee.

### Procedure

For the first 3 d the rats were fed on a chow diet and given water *ad libitum* in their individual cages where all experiments took place (habituation period). During the following 8 d the rats were pre-fed with one of the semi-purified diets depending on the experiment (pre-feeding period). During the next 4–8 d all rats were given a choice between two diets (self-selection period). The various diets are listed later and described in detail in Table 1. Both feed containers were refilled with fresh feed and their placement on each side of the cage was alternated daily to prevent side-choice effects. Feed consumption and body weight were measured daily at 17.00 hours. In Expts 2c and 3, during the first choice day, feed consumption was measured hourly. All the diets were moistened (water-powdered diet, 1:2) to prevent spillage. Water was available continuously.

Table 1. *Composition of the diets used in the experiments (g/kg diet)*

Diet...	P6%	P20%	P0%	COR	THR-DEV	ILE-DEV	TRP-DEV
Casein*	60	200	—	—	—	—	—
Non-essential amino acids†	—	—	—	77	77	77	77
Essential amino acids‡	—	—	—	130	124	115	126
L-Methionine	0.5	1.5	—	—	—	—	—
Salt mixture§	45	45	45	45	45	45	45
Vitamin mixture	10	10	10	10	10	10	10
African peanut oil¶	20	20	20	20	20	20	10
Rapeseed oil¶¶	30	30	30	30	30	30	30
Cellulose**	20	20	20	20	20	20	20
Maize starch††	407.2	336.7	437.5	334.0	337.0	341.5	336.0
Glucose‡‡	407.2	336.7	437.5	334.0	337.0	341.5	336.0
Total§§	1000	1000	1000	1000	1000	1000	1000

\*Vitamin-free, ICN Biomedicals, OH, USA.

†Provided (g/kg diet): glutamic acid 30, L-glycine 10, L-arginine 10, L-alanine 3.5, L-asparagine 10, L-proline 10, L-serine 3.5, total 77 (Gietzen *et al.* 1992).

‡Provided (g/kg diet): THR-DEV: L-methionine 10, L-cystine 6, L-histidine 12, L-lysine 15, L-isoleucine 15, L-leucine 21, L-phenylalanine 15.5, L-tryptophan 4, L-valine 16, L-tyrosine 9.5, total 124; ILE-DEV: L-methionine 10, L-cystine 6, L-histidine 12, L-lysine 15, L-threonine 6, L-leucine 21, L-phenylalanine 15.5, L-tryptophan 4, L-valine 16, L-tyrosine 9.5, total 115; TRP-DEV: L-methionine 10, L-cystine 6, L-histidine 12, L-lysine 15, L-threonine 6, L-isoleucine 15, L-leucine 21, L-phenylalanine 15.5, L-valine 16, L-tyrosine 9.5, total 126; COR: L-methionine 10, L-cystine 6, L-histidine 12, L-lysine 15, L-threonine 6, L-isoleucine 15, L-leucine 21, L-phenylalanine 15.5, L-tryptophan 4, L-valine 16, L-tyrosine 9.5, total 130 (Gietzen *et al.* 1992). All amino acids were purchased from Degussa Corporation Chemical Group, Ridgefield Park, NJ, USA.

§Provided (g/kg diet): calcium phosphate 17.1, potassium phosphate 10.8, calcium carbonate 8.1, magnesium sulphate 4.0, sodium chloride 3.16, magnesium oxide 0.9, ferric sulphate 0.39, zinc sulphate 0.23, manganese sulphate 0.23, cupric sulphate 0.046, sodium fluoride 0.037, potassium chromate 0.002, potassium iodide 0.002, ammonium molybdate 0.001, cobalt carbonate 0.001, sodium selenite 0.001.

||Provided (per kg diet): retinyl acetate 5 mg, cholecalciferol 62.5 µg, DL- $\alpha$ -tocopherol acetate 5 g, menadione 1 mg, thiamin 10 mg, riboflavin 10 mg, nicotinic acid 45 mg, D-calcium pantothenate 30 mg, pyridoxine-HCl 10 mg, inositol 50 mg, D-biotin 0.2 mg, pteroylmonoglutamic acid 2 mg, cyanocobalamin 0.0135 mg, ascorbic acid 100 mg, *p*-amino benzoic acid 50 mg, choline chloride 750 mg.

¶Lesieur Aliment, BP889, 59412 Coudekerque Branche, France.

\*\*Medias Filtrants Durieux, BP 59, ZI Torey 7702, France.

††Cerestar, BP109, 59482 Hanbourdin cedex, France.

‡‡Roquette Frères, 4 rue Patou, 59022 Lille, France.

§§All ingredients were purchased or prepared by the atelier de préparation des aliments expérimentaux, Institute National de la Recherche Agronomique, Jouy en Josas, 78 France.

### *Composition of diets*

The composition of the diets is shown in Table 1. Briefly, according to Centre National d'Etudes et de Recommandations pour la Nutrition et l'Alimentation (CNERNA) recommendations (Potier de Courcy *et al.* 1989), all of the diets contained (g/kg): cellulose 20, lipids 50, (rapeseed oil 30, and peanut oil 20), mineral mixture 45, and vitamin mixture 10. Carbohydrate varied between 640 and 820 g/kg DM and the remainder was made up of protein. The carbohydrate fraction was a mixture of equal parts of maize starch and glucose adjusted to make 100% of the DM. The specific composition of the various diets was as follows:

P0%: protein-free diet;

P6%: a diet containing 60 g casein/kg;

P20%: a diet containing 200 g casein/kg;

THR-DEV: a diet devoid of threonine, but well balanced for the rest of the essential amino acids (Gietzen *et al.* 1992);

ILE-DEV: a diet devoid of isoleucine (instead of threonine) but otherwise well balanced for essential amino acids;

TRP-DEV: a diet devoid of tryptophan (instead of threonine) but otherwise well balanced for essential amino acids (the ILE-DEV, TRP-DEV, and THR-DEV diets were identical except for the deficient amino acid);

COR: a corrected diet. This diet was identical to the threonine-devoid diet, but supplemented with 6 g threonine/kg in order to correct the balance of the amino acid mixture. The COR diet was used throughout these experiments because it was fully balanced for all of the essential amino acids and, thus was replete with each of the limiting amino acids used in the DEV preparations. The COR diet appeared to have similar oro-sensory qualities to the DEV diets.

### *Data analysis*

Except when mentioned, the outcome variable was the COR preference ratio, calculated as the grams of COR diet intake divided by total feed intake (Booth, 1987). A 'COR preference' was assumed when the COR preference ratio was more than 0.5 and statistically different from 'no choice' as indicated by a ratio of 0.5. The expression 'clear COR preference' refers to a COR preference ratio of  $> 0.75$ . All results, e.g. preference ratio, are shown as means with their standard errors (SEM). Statistical significance was determined by ANOVA using STAT-GRAPH (Statistical Graphic Corporation, Rockville, MD, USA), introducing diets, days and groups as factors. Statistical significance was set at  $P < 0.05$ . When differences were detected by ANOVA, differences between individual means were determined using a post-hoc test (Scheffé  $P < 0.05$ ).

#### RESULTS: EXPT 1. CHOICE BETWEEN A THREONINE-DEVOID DIET AND A THREONINE-CORRECTED DIET IN NORMAL AND AMINO ACID-DEFICIENT RATS

When given a choice, both thiamin-deficient and thiamin-recovered rats, but not normal rats, choose the thiamin-replete diet over the familiar deficient one (Scott & Verney, 1947, 1948; Rozin *et al.* 1964; Rozin, 1965). Moreover, as rats recover from the thiamin deficiency and no longer need thiamin, they still show a clear thiamin preference in the same proportion as thiamin-deficient rats do; Rozin concluded that a current need for the corrected diet is not necessary to induce a choice in its favour. Past history of thiamin deficiency seems to be a sufficient condition for the development of thiamin preference.

We asked the question whether preference for an amino acid-replete diet would be similarly conditioned with amino acid deficiency or imbalance. The purpose of Expt 1a was to determine the influence of a threonine deficiency, and the consequent induced aversion, on the daily choice pattern between a familiar threonine-deficient diet and a similar but now corrected diet.

When faced with a choice between two novel diets or two versions of a novel diet, one of them being thiamin-deficient, naïve rats are unable to choose the appropriate diet (Rodgers, 1967). As previously mentioned, Leung *et al.* (1968) have shown that naïve rats prefer a threonine-corrected diet over a threonine-imbalanced diet and that the preference for the adequate diet seems to begin on the very first choice day. Leung & Rogers (1985) evaluated the choice between an amino acid-devoid diet and the corresponding corrected diet. However, in that experiment rats were not really naïve because they had tried both of the diets in previous choices. In spite of that, during the first day (even if both diets were not novel), according to our recalculation the preference ratio for the corrected diet was only 0.55. From the data of Leung & Rogers (1985) it is not clear whether this preference ratio was statistically different from no choice (0.5). After several days (the sixth day results were shown in the paper) a clear preference for the corrected diet was established (0.89). Experiment 1b aimed to find out when, in our experimental conditions, the naïve rat chooses between two versions of a novel diet, one of the them being threonine-deficient. Expt 1c was the same as Expt 1b, using isoleucine, another essential amino acid, as the limiting amino acid.

*Expt 1a. Daily choice by amino acid-deficient rats given two versions of the familiar deficient diet, one of them being corrected*

The purpose of this experiment was to determine how many days threonine-deficient rats need to make a clear choice between two versions of the familiar threonine-deficient diet, one corrected.

For 8 d, twelve male albino rats (235–260 g initially) were pre-fed with the THR-DEV diet. During the following 4 d they were given a choice between a THR-DEV diet and a COR diet.

A clear COR preference began on the first choice day (0.77, SEM 0.11) and was different from no choice ( $P=0.0026$ ). During the four choice days the COR preference ratio remained stable with a clear overall COR preference measured (+0.79, SEM 0.04; Fig. 1).

*Expt 1b. Daily choice by naïve rats between two versions of a novel diet, one of them being threonine-deficient*

The purpose of this experiment was to determine how many days naïve rats, pre-fed with a balanced low-protein diet, need to make a clear choice between two versions of a novel diet, one threonine-deficient and the other corrected.

For 10 d twenty-six male albino rats (220–320 g initially) were pre-fed with the P6% diet. During the following 8 d they were given a choice between the THR-DEV diet and the COR diet.

The COR preference began on the first day (0.575, SEM 0.023) and was different from 0.5 which indicates no choice ( $P=0.035$ ). During the first 4 d of choice the COR preference ratio increased day after day reaching thereafter a plateau ( $F(3,100)$  7.13,  $P=0.0002$ ; Fig. 1). The average COR preference ratio over the first four choice days was +0.68 (SEM 0.06). A between-group comparison indicated a difference ( $F(1,148)$  14.45,

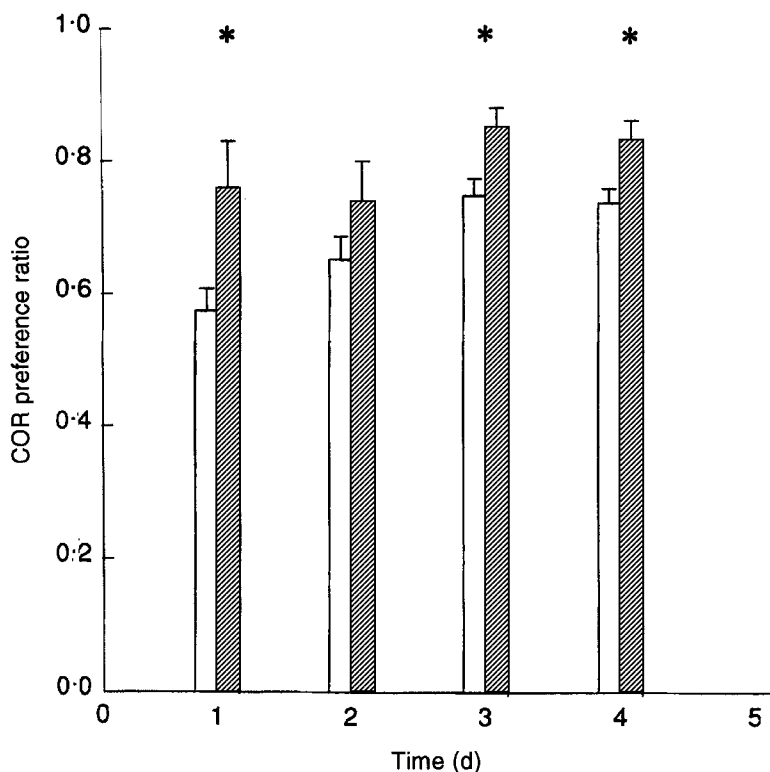


Fig. 1. Preference of rats for the corrected diet when given a choice between two versions of a diet, one threonine-deficient and the other corrected (COR). The COR preference ratio is the intake of the COR diet/total intake. The choice period began on day 1. The results of two experiments are shown. In Expt 1a (▨) rats were pre-fed on a diet devoid of threonine until day 0; in Expt 1b (□) rats were pre-fed on a low-protein diet until day 0. For details of diets and procedures, see Table 1 and pp. 300–302. \*Values from the two experiments were significantly different ( $F(1,148)$  14.45,  $P < 0.0002$ ).

$P = 0.0002$ ) between Expts 1a (pre-feeding diet: THR-DEV) and 1b (pre-feeding diet: P6%).

*Expt 1c. Daily choice by naïve rats between two versions of a novel diet, one of them being isoleucine-deficient*

The purpose of this experiment was to compare the results obtained using isoleucine as the limiting amino acid with those with the THR-DEV diet, in rats pre-fed with the P6% diet.

This experiment was identical to Expt 1b except that the ILE-DEV diet was used instead of the THR-DEV diet. For 10d twelve male albino rats (260–270 g initially) were pre-fed with the P6% diet. During the following 4 d they were given a choice between the ILE-DEV diet and the COR diet.

The COR preference began on the very first choice day (0.60, SEM 0.11) and was different from no choice ( $P = 0.046$ ). As early as the second choice day a clear COR preference was measured (0.8, SEM 0.05, Fig. 2) and then remained stable. Except for the first choice day, a between-group comparison indicated a difference ( $F(1,144)$  8.9,

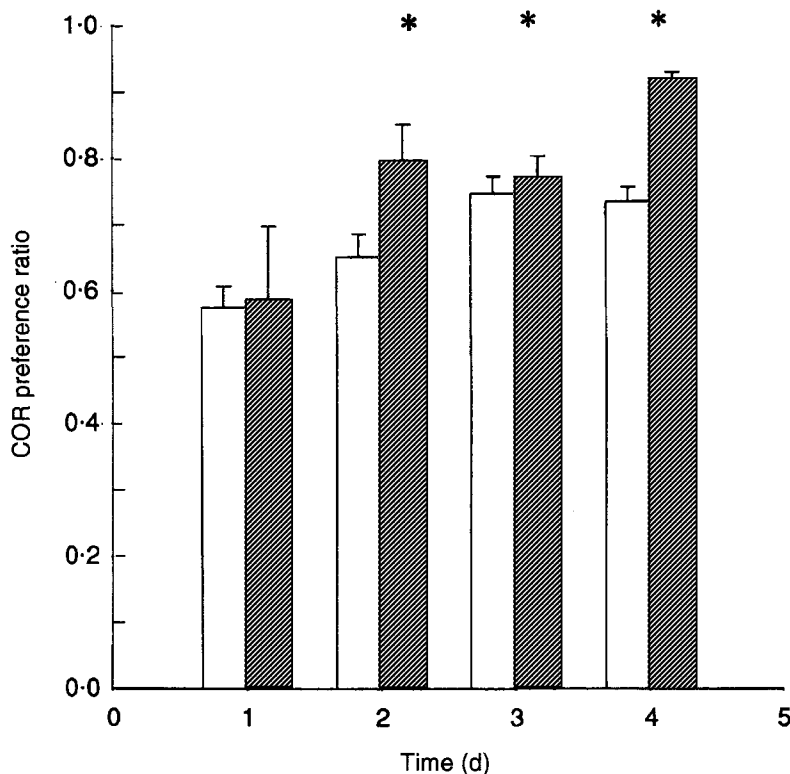


Fig. 2. Preference of rats for the corrected diet when given a choice between two versions of a diet, one amino acid-deficient and the other corrected (COR). The COR preference ratio is the intake of the COR diet/total intake. The choice period began on day 1. The results of two experiments are shown. In Expt 1b (□) the choice was between the COR diet and a diet devoid of threonine. In Expt 1c (▨) the choice was between the COR diet and a diet devoid of isoleucine. \*Values from the two experiments were significantly different ( $F(1,144)$  8.9,  $P=0.03$ ).

$P=0.03$ ) between Expts 1b (choice between COR and THR-DEV) and 1c (choice between COR and ILE-DEV).

**Discussion.** In both the thiamin (Rozin, 1965) and the amino acid cases, during the first four choice days deficient rats showed an overall avoidance of the familiar deficient version of the diet or an overall preference for the well-balanced version, or both. Amino acid-deficient rats showed a clear preference for the well-balanced version as early as the first choice day whilst in the case of thiamin deficiency, less than half of the thiamin-deficient rats showed a preference for the adequate version during the first two choice days (Rozin, 1965).

The present results showed that in the case of amino acid deficiencies, well-balanced rats needed just one day to make an adequate choice between two versions of a novel diet, one of them being devoid of an amino acid. After the second day in the case of isoleucine and the third day in the case of threonine, rats established a clear COR preference (preference ratio  $< 0.75$ ). In contrast, in the case of thiamin deficiency, normal rats failed to choose the adequate diet even after four choice days (Rozin *et al.* 1964). During the first choice day the nature of the amino acid deficiency had no influence on the COR preference ratio. However, after the first choice day rats chose the COR diet more easily with isoleucine deficiency than with threonine deficiency.

This time difference may be due to the time necessary to develop a deficiency for the nutrient in previously well-nourished rats. It generally takes several days (at least 8 d) to develop a thiamin-deficient state in previously well-nourished rats (Rozin *et al.* 1964). In contrast, essential amino acid deficiency occurs within a few hours (Harper *et al.* 1970) because there are no storage sites for essential amino acids; those not used for protein or other biosynthesis are catabolized.

RESULTS: EXPT 2. CHOICE BETWEEN A PROTEIN-FREE DIET AND A PREVIOUSLY AMINO ACID-DEFICIENT BUT NOW CORRECTED DIET, IN AMINO ACID-DEFICIENT RATS

Rozin (1967) demonstrated that thiamin deficient feed becomes aversive to thiamin-deficient rats. Specific aversion is demonstrated by spillage, redirected feeding responses and hypophagia. Both thiamin-deficient and thiamin-recovered rats, but not naïve rats, prefer a novel feed, even a deficient one, rather than the familiar deficient but now corrected one (Rozin & Rodgers, 1966, Rodgers & Rozin, 1967). From these experiments the authors emphasized the importance of the aversion in the latter choice and in the induced non-specific neophilia. Only 4 d later, rats reverse their initially adverse choice and thereafter prefer the thiamin-corrected diet. This reversal of choice seems to be due to an induced aversion for the novel deficient diet.

Specific aversive behaviours have long been observed in rats fed on amino acid-imbalanced or deficient diets (Rogers & Leung, 1972). Moreover, from the very first day, according to our recalculation, naïve rats show a clear preference ( $> 0.75$ ) for a corrected diet over a protein-free diet (Leung *et al.* 1968). As rats rapidly recognize the metabolic effects of amino acid-deficient diets (Gietzen *et al.* 1986), one could expect that the induced aversion in amino acid-deficient rats would be very rapidly reversed on feeding the COR diet, perhaps more rapidly than in the case of thiamin-deficient rats.

Three experiments were performed to characterize, under our experimental conditions, how the amino acid-deficient rat would make an adequate choice between a protein-free diet and a previously devoid but now threonine-corrected diet. To compare the results obtained with earlier work using thiamin, a protein-free diet was used as a novel deficient diet. Three experimental trials were done: the daily choice between a corrected diet and a protein-free diet in naïve rats (Expt 2a), in threonine-deficient rats (Expt 2b) and, using another essential amino acid, in tryptophan-deficient rats (Expt 2c).

*Expt 2a. Daily choice by naïve rats given a corrected diet and a protein-free diet*

The purpose of this experiment was to assess the capacity of naïve rats to choose between the COR diet and the P0% diet.

For 10 d twelve male albino rats (235–320 g initially) were pre-fed with the P6% diet. During the following 6 d they were given a choice between the P0% diet and the COR diet.

Only on the fourth choice day was the COR preference significantly different from no choice (0.63, SEM 0.06;  $P = 0.012$ ). During the entire 6 d choice period the COR preference ratio increased day after day but the daily variations were not significant (Fig. 3).

*Expt 2b. Pattern of the choice by threonine-deficient rats given a corrected diet and a protein-free diet*

The purpose of this experiment was to determine (1) how many days threonine-deficient rats need to reverse the acquired aversion when given a choice between a protein-free diet, used as a novel deficient diet, and a familiar previously threonine-devoid but now



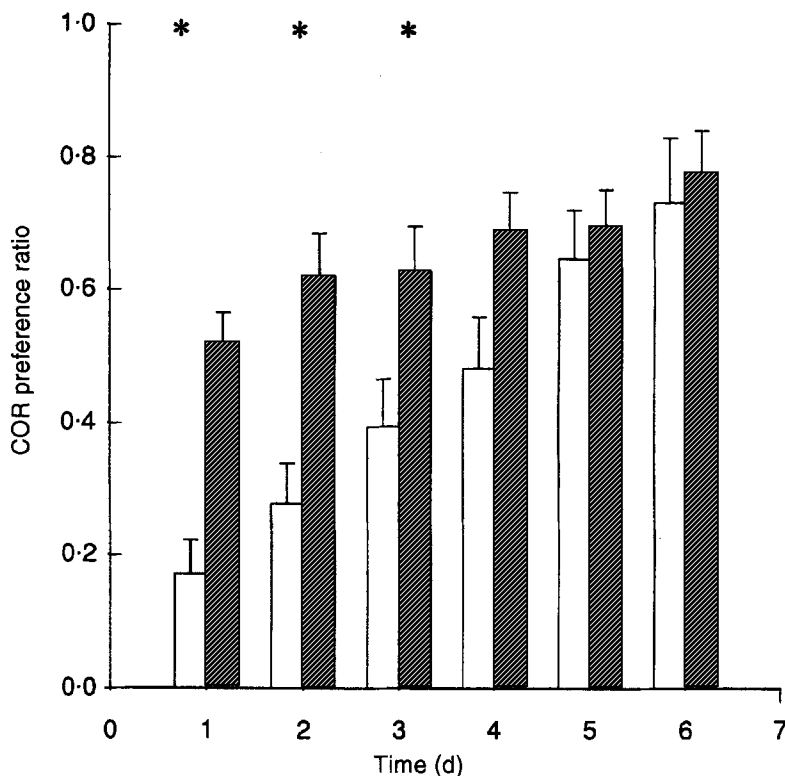


Fig. 3. Preference of rats for the corrected diet when given the choice between a protein-free diet and a corrected diet (COR). The COR preference ratio is the intake of the COR diet/total intake. The choice period began on day 1. The results of two experiments are shown. In Expt 2a (▨) rats were pre-fed on a low-protein diet until day 0; in Expt 2b (□) rats were pre-fed on a diet devoid of threonine until day 0. For details of diets and procedures, see Table 1 and pp. 300–302, 306–307. \*Values from the two experiments were significantly different ( $F(1,107)$  35.17,  $P < 0.0001$ ).

corrected diet; (2) to compare their daily performance with that of naïve rats, only moderately protein-deprived (as assessed in Expt 2a); and (3) to compare later their hourly and daily performance with that of protein-deficient rats, when given a choice between the familiar protein-free diet and the THR-DEV diet used as a novel deficient diet (as assessed in Expt 3).

For 8 d eighteen male albino rats (205–330 g initially) were pre-fed with the THR-DEV diet. During the following 6 d they were given a choice between the previously threonine-devoid but now corrected diet and the P0% diet. Furthermore, during the first choice day the feed intakes were measured 1 and 5 h after the start of the choice.

As early as the first choice hour a preference (0.98, SEM 0.20) for the novel protein-deficient diet (P0%) was observed (Table 2). During the first two choice days rats preferred the P0% diet rather than the COR diet. However, during the first five choice days the COR preference ratio increased ( $F(5,74)$  7.23,  $P < 0.001$ ), (Fig. 3). After the fifth choice day the COR preference was greater than 0.5 (0.65, SEM 0.07;  $P = 0.03$ ) and then remained stable. During the first three choice days a between-group comparison indicated a difference ( $F(1,107)$  35.17,  $P < 0.0001$ ) between Expts 2a (pre-feeding P6% diet) and 2b (pre-feeding THR-DEV diet), but during the following three choice days the COR preference ratios were not different.

Table 2. Cumulative feed intakes (kJ) and preference ratios for rats given a choice of two diets after being pre-fed on a diet devoid of threonine (THR-DEV) or a protein-free diet (P0%)†

(Mean values with their standard errors; values are for the first two choice days after pre-feeding)

Time (h) ...	1		2		5		24		48	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 2b: COR v. P0% (pre-fed THR-DEV)										
COR (kJ)	0.84	0.84	3.35	1.67	10.88	5.02	22.60	10.34	154.34	48.14
P0% (kJ)	35.75	1.72	64.63	15.28	125.50	9.59	324.87	18.18	200.51	48.98
P0% : total	0.98† <sup>a</sup>	0.02	0.92† <sup>a</sup>	0.04	0.93† <sup>a</sup>	0.03	0.94† <sup>a</sup>	0.03	0.59 <sup>b</sup>	0.13
Expt 3: P0% v. THR-DEV (pre-fed P0%)										
P0% (kJ)	8.54	3.26	18.17	6.36	44.66	19.05	203.98	33.74	279.33	22.80
THR-DEV (kJ)	8.04	3.64	19.72	9.96	32.99	11.18	66.68	7.66	47.89	20.34
DEV : total *	0.48	0.15	0.52	0.09	0.42†	0.13	0.32†	0.10	0.14†	0.05

COR, diet corrected for an amino acid deficiency.

<sup>a,b</sup>Mean values within a row with unlike superscript letters were significantly different ( $F(4,25)$  5.80,  $P < 0.0019$ ).

\*Mean values were significantly different from the P0% : total values in Expt 2b ( $F(1,58)$  54.95,  $P < 0.0001$ ).

†Mean values were significantly different from no choice (ratio of 0.5) ( $P < 0.05$ ).

‡For details of diets and procedures, see Table 1 and pp. 300–302, 306–310.

*Expt 2c. Pattern of choice by tryptophan-deficient rats given a corrected diet and a protein-free diet*

The purpose of this experiment was to compare the results obtained in threonine deficient rats with those in tryptophan-deficient rats. Tryptophan is an essential amino acid in the diet. It is often the amino acid in the lowest concentration in proteins; it also serves as a precursor of serotonin. Therefore, an aversion in tryptophan-deficient rats might be very rapidly reversed, perhaps more rapidly than in the case of threonine deficiency.

For 8 d six male albino rats (205–330 g initially) were pre-fed with the TRP-DEV diet. During the following 5 d they were given a choice between the COR diet, with oro-sensory properties similar to the previously fed TRP-DEV diet, and a P0% diet.

During the two first choice days rats preferred the P0% diet rather than the COR diet (Fig. 4). However, during the next five choice days the COR preference ratio increased ( $F(3,20) 6.34, P=0.003$ ). After the fifth day the COR preference ratio (0.60, SEM 0.05) was still above 0.5 ( $P=0.043$ ). A between-group comparison indicated no difference between Expts 2b (choice between a COR diet and a THR-DEV diet) and 2c (choice between a COR diet and a TRP-DEV diet).

*Discussion.* During the first days naïve rats showed a preference ratio for the corrected diet that was less clear cut in our experiment than that seen in Leung's experiment (Leung

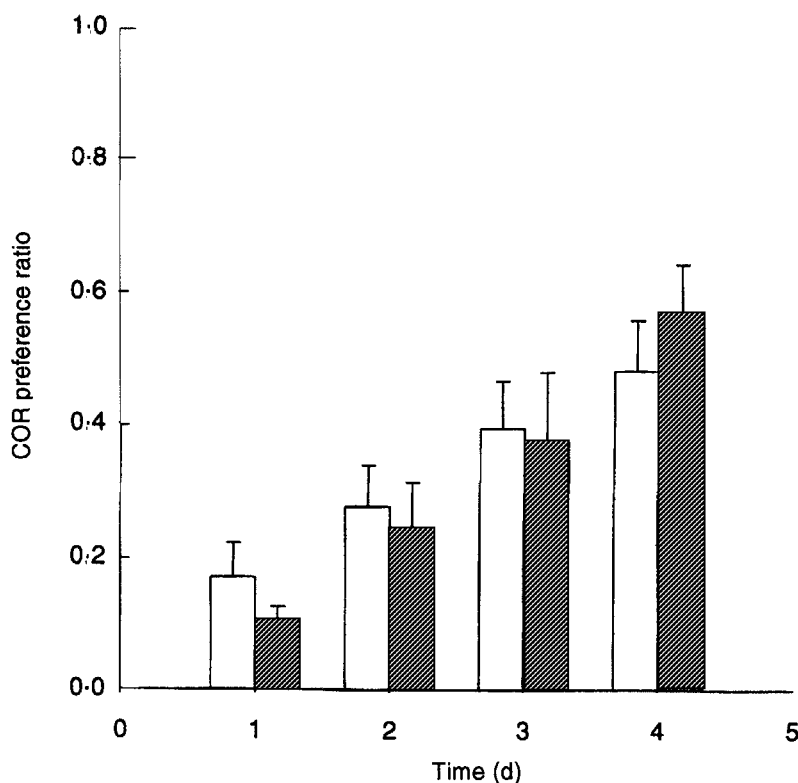


Fig. 4. Preference of rats for the corrected diet when given a choice between a protein-free diet and a corrected diet (COR). The COR preference ratio is the intake of COR diet/total intake. The choice period began on day 1. The results of two experiments are shown. In Expt 2b (□) rats were pre-fed on a diet devoid of threonine; in Expt 2c (▨) rats were pre-fed on a diet devoid of tryptophan. For details of diets and procedures, see Table 1 and pp. 300–302, 306–309.

*et al.* 1968). Such a difference could be due to the difference in level of protein of the corrected diets in the two experiments (respectively 207 and 115 g/kg of diet for our experiment and Leung's). In the present experiment, when rats ate a mixture of the corrected and the protein-free diets (for instance 50–50 on the first choice day) the level of protein intake would meet the requirement for an adult rat from both the quantitative and the qualitative point of view. In the study of Leung *et al.* (1968) the level of protein in the corrected diet was certainly lower than ours and closer to published protein requirements. Thus, in Leung *et al.* (1968) experiment any mixture would provide less than the required protein intake in contrast to the various mixtures in our experiments, which resulted in a slight excess. Both threonine- and tryptophan-deficient rats showed a preference for the novel deficient diet rather than for an amino acid-corrected diet, the oro-sensory properties of which were similar to the previous devoid diet. As in the case of thiamin deficiency (Rozin & Rodgers, 1966), in amino acid-deficient rats, 4 d are necessary to obtain a preference for the corrected diet. After 8 d on an amino acid-deficient diet, rats had clearly developed an aversion to this familiar diet. The present results indicate that a non-specific neophilia may be induced by the aversion to the familiar amino acid-deficient diet. Alternatively, if the COR and DEV diets had similar oro-sensory properties, the aversion to the DEV diet could have carried over to the COR diet, and it may have taken several days to extinguish this learned aversion and eat enough of the COR diet to experience its positive metabolic effects. However, a question remains: what is the nature of the preference for the protein-free diet? Harper *et al.* (1970) suggested that rats prefer a protein-free diet over an amino acid-imbalanced diet because the plasma amino acid imbalance is less with a protein-free diet.

#### RESULTS: EXPT 3. EFFECTS OF PREVIOUS AVERSION IN EITHER PROTEIN- OR THREONINE-DEFICIENT RATS

In the past, choice experiments have been conducted in protein-deficient rats (Sanahuja & Harper, 1962; Leung *et al.* 1968). Rats, pre-fed on a protein-free diet, were given a choice between a protein-free diet and an amino acid-imbalanced diet. As a protein-free diet also induces an aversion, it would be expected that if there was a non-specific neophilia, rats would prefer the novel imbalanced diet rather than the protein-free diet to which they had developed an aversion. The results of the two experiments listed previously were not consistent. In the experiment of Sanahuja & Harper (1962), as early as the first choice day a preference for the amino acid-imbalanced diet was obtained and maintained for at least 3 d, whereas in the experiment of Leung *et al.* (1968), rats maintained a preference for the protein-free diet. The purpose of Expt 3 was to determine, under our experimental conditions, which diet protein-deficient rats would choose if given a choice between the familiar protein-free diet and a THR-DEV diet used as a novel deficient diet.

For 8 d six male albino rats (245–260 g initially) were pre-fed with the P0% diet. During the following 2 d they were given a choice between the THR-DEV diet and the P0% diet. The THR-DEV preference ratio was calculated as THR-DEV intake/total feed intake. Furthermore, during the first choice day the feed intakes were measured 1 and 5 h after the start of the choice.

During at least the first 2 h of choice the rats ate the same amount of the two diets (Table 2). However, no preference for the novel THR-DEV diet was observed. As early as the first choice day the rats preferred the P0% diet (the THR-DEV preference ratio was 0.23 (SEM 0.01)). An overall between-group comparison indicated a difference between Expts 2b (choice between a COR diet and a THR-DEV diet) and 3 (choice between a P0% diet and a THR-DEV diet) ( $F(1,58) 54.995, P < 0.0001$ ).

*Discussion.* The results reported by Leung *et al.* (1968) are supported by the present experiment, although in our experiment we used an amino acid-devoid diet as opposed to the amino acid-imbalanced diet used in Leung's experiment. Although our Expt 2b suggested that, as with thiamin deficiency, an amino-acid induced aversion does favour neophilia, this neophilia shows more subtle properties when a choice of a novel deficient diet is offered. Expt 3 showed that the protein-deficient rat preserves its capacity to discriminate among the diets offered rather than preferring any new diet. That this choice was not due to a better taste of the protein-free diet, compared with the devoid diet, is supported by the data of Sanahuja & Harper (1962), who showed that, as early as the very first day, protein-deficient rats prefer a corrected diet containing free amino acids like our diets, rather than a protein-free diet. In contrast to the thiamin-deficient rats, we suggest that protein-deficient rats express another type of neophilia that is not non-specific.

In Expt 3 we used protein-deficient rats instead of amino acid-deficient rats which were widely used as a model of protein deficiency in the 1960s (Sidransky & Miloslav Rechcigl, 1962; Said *et al.* 1974). The short- and the long-term consequences of ingesting a threonine-, or an isoleucine-, or a total sulphur-amino-acid-deficient diet are very similar to those induced by a protein-deficient diet, i.e. decreased feed intake, body weight and, eventually, death. Comparing the results of Expt 2b, in animals averse to a threonine devoid diet, and Expt 3, animals averse to a protein-free diet, preferences were not similar. As with essential amino-acid imbalance, rats, even if they were already averse to the protein-free diet, preferred that protein-free diet to a threonine-devoid diet. This suggests that an essential specific amino acid-devoid diet is more aversive than a protein-devoid diet.

#### GENERAL DISCUSSION

These experiments extend previous data on amino acid selection and allow comparisons with the earlier literature on thiamin deficiency. Among the similarities: first, both thiamin- and amino acid-deficient rats acquire an aversion for the familiar deficient feed and consequently a neophilia for a novel beneficial feed when available (Rozin, 1965; Rozin & Rodgers, 1966; Rodgers & Rozin, 1967 and results of Expt 2). Second, in both cases a delay of at least 4 d is necessary to reverse the initial choice in favour of the novel deficient feed, to extinguish any residual learned aversion to the familiar feed, and to prefer the now corrected familiar version (Rozin & Rodgers, 1966, and results of Expt 2). Expt 2 shows that, as in thiamin deficiency, the amino acid-induced aversion favours neophilia. It is likely that rats were confused by the similar oro-sensory properties of the two amino acid diets. Moreover, rats were naïve with respect to the metabolic consequences of both versions that were not equivalent. When choosing for the first time between the novel deficient diet and the previously deficient but now corrected diet, amino acid-deficient rats probably avoided the latter because of the oro-sensory identity of the two versions of the amino acid diet.

There are important differences, however, between the responses to amino acid deficiency and thiamin deficiency. First, both thiamin- and amino acid-deficient rats show, certainly, a preference for the well balanced version (Rozin, 1965; results of Expt 1a) but it is more clear-cut in amino acid deficiencies than in thiamin deficiency. Second, 1 d is enough for amino acid-replete rats to choose between two versions of a novel diet, one of them being devoid of an essential amino acid (Expt 1). In the case of thiamin and of several minerals like Ca and K, well-nourished rats do not choose between two versions of a novel diet when one of them is micronutrient deficient (Rodgers, 1967; Rozin, 1976). This

finding may be accounted for by differences in storage pools. Both thiamin- and mineral-deficient feeds are tolerated much longer before being identified as deleterious and therefore aversive because of substantial body stores of these components. In contrast, essential amino acids have no storage pool. Thus, essential amino acid imbalances and deficiencies induce rapid detection and avoidance of the diet that has been identified by the subject as being responsible for the metabolic effects. The orders of magnitude for these delays are days *v.* hours, for thiamin *v.* amino acids respectively. The difference in delay might also be due to the presence of some sort of gastrointestinal sensing of individual amino acids, a mechanism that seems to be discarded in the case of B-vitamins.

The third main difference is that protein-deficient rats exhibit a non-specific neophilia that is peculiar. Even if they show a neophilia as assessed by the first hour's choice in Expt 3, subsequently they still continue to select the aversive familiar diet (P0%) when the novel diet is devoid of one amino acid; as if in protein-deficient rats neophilia were accompanied by an increased distrustfulness that allows them to detect that the novel diet is in fact inappropriate and therefore not better. In that respect, equivalent metabolic and pathologic consequences observed when rats eat a protein-free diet or amino acid-deficient diets were recalled earlier; rats routinely choose a protein-free diet over an amino acid-deficient diet, whether imbalanced or devoid (Leung & Rogers, 1985). Amino acid deficiency could induce alertness either by reducing the threshold of detection or by increasing the signal : noise ratio and so exacerbating the degree of attention towards the cue. Hrupka *et al.* (1994) reported that lysine-deficient, but not lysine-replete, rats were able to detect a few parts per million of lysine in a dietary choice. While it is possible that protein-deficient rats increase their ability to discriminate the protein balance of a diet more easily than both well balanced and amino acid-deficient rats, the differences between the results of Expts 2b and 3 are probably due to the time required for the extinction of the learned aversion in rats pre-fed with THR-DEV *v.* the time to detect the adverse effects of THR-DEV in rats pre-fed on the protein-free diet.

Rats, and perhaps all omnivores, may use other additional strategies for adapting their aversions and preferences. Beyond the expression of neophilia alone (Rozin, 1965), there might be shifts of sensory mechanisms as well. If a deficient animal were unable to show the propensity to consume novel foods, the chance of encountering an adequate one would be less. Neophilia aims at encountering new and perhaps beneficial food items. Alternatively, neophobia serves as a parallel strategy by questioning a novel food's safety. Induced neophobia favours the rejection of any novel food, or at least induces increased caution about the adequacy of the novel food. Although a little paradoxical, it is possible that deficient rats show a concomitant enhancement of both their neophilia and neophobia. These two concomitant patterns may account for the daily changes observed in our experiments. Because the choice for the same diets varied among the experiments and over time, depending on the pre-feeding diet and the nutritional consequences, no preferences based on the pre-ingestive qualities of the diets could be determined.

Beneficial ingestants can be detected either by innate or learned mechanisms. Both are enhanced when animals are in a critical need dependent state (Baker *et al.* 1987). In the case of an innate detection system, such as detection of a salty taste which is enhanced in Na-deficient subjects, the recognition of the beneficial item is immediate and so is the preference (Richter, 1956; Rodgers, 1967). If the discrimination is not innate and has to be learned more or less laboriously (depending on its salience, novelty, signal : noise ratio), preference itself will be more or less delayed, taking into account the oro-sensory characteristics and the post-absorptive consequences of ingesting the food, along with the effects of previous conditioning. Once established, the learned discrimination will tend to

become as efficient as the innate one. Deutsch and his co-workers (Deutsch *et al.* 1989; Heinrichs *et al.* 1990) reported that severely protein-depleted rats exhibit an unlearned specific appetite for certain proteins and a preference for protein-associated odours. Unfortunately, the design of their previous sessions with protein-containing v. protein-free diets may have induced an associative learned preference for some 'protein odours' (Heinrichs *et al.* 1990). Hitherto, there is no unequivocal demonstration of a taste-odour-bound detection system for essential amino acids. As for the umami taste, it is proposed to be an index of protein in general rather than an index of one specific component, essential or not, of the protein (Viарouge *et al.* 1991). Our results show that threonine-deficient rats fail to indicate an immediate preference for the corrected diet, in the first few hours. We therefore conclude that the appetite for threonine-containing diets is apparently not an innate but a learned appetite.

The results available from the literature together with the present results shed more light on the subtle mechanisms that allow optimization of the choices in omnivores. When an animal's nutritional state starts becoming imbalanced, it develops new strategies, or exacerbates pre-existing ones, that lead to a larger array of choices (neophilia) together with a more accurate detectability and switch of preferences, even towards foods that were previously aversive. However, such a switch of preference is accompanied by an increased distrustfulness and possibly by an increased sensory discriminability that we refer to as neophobia. Thus, a concomitant enhancement of neophilia and neophobia is not incompatible. It only results in an optimal meal strategy for the choice of the most appropriate foods.

#### REFERENCES

- Baker, B. J., Booth, D. A., Duggan, J. P. & Gibson, E. L. (1987). Protein appetite demonstrated: learned specificity of protein-cue preference to protein need in adult rats. *Nutrition Research* 7, 481-487.
- Booth, D. A. (1985). Food-conditioned eating preferences and aversions with interoceptive elements: conditioned appetites and satieties. *Annals of the New York Academy of Sciences* 443, 23-41.
- Booth, D. A. (1987). How to measure learned control of food or water intake. In *Feeding and Drinking*, pp. 111-149 [F. M. Toates and N. E. Rowland, editors]. London: Elsevier.
- Deutsch, J. A., Moore, B. A. & Heinrichs, S. C. (1989). Unlearned specific appetite for protein. *Physiology and Behavior* 46, 619-624.
- Gietzen, D. W. (1993). Neural mechanisms in the responses to amino acid deficiency. *Journal of Nutrition* 123, 610-625.
- Gietzen, D. W., Leung, P. M. B., Castonguay, T. W., Hartmann, W. J. & Rogers, Q. R. (1986). Time course of food intake and plasma amino acid concentrations in rats fed amino acid imbalanced or deficient diets. In *Interaction of the Chemical Senses with Nutrition*, pp. 415-456 [M. R. Kare and J. Brand, editors]. New York: Academic Press.
- Gietzen, D. W., McArthur, L. H., Thiesen, J. C. & Rogers, Q. R. (1992). Learned preference for the limiting amino acid in rats fed a threonine-deficient diet. *Physiology and Behavior* 51, 909-914.
- Halstead, W. C. & Gallagher, B. (1962). Auto regulation of amino acids in the albino rat. *Journal of Comparative and Physiological Psychology* 55, 107-111.
- Harper, A. E., Benevenga, N. J. & Wohlhueter, R. M. (1970). Effects of ingestion of disproportionate amounts of amino acids. *Physiological Reviews* 80, 428-458.
- Harris, L. J., Hargreaves, F. & Ward, A. (1933). Appetite and choice of diet. The ability of the vitamin B deficient rat to discriminate between diets containing and lacking the vitamin. *Proceedings of the Royal Society of London, Series B* 113, 161-190.
- Heinrichs, S. C., Deutsch, J. A. & Moore, B. E. (1990). Olfactory self-selection of protein containing foods. *Physiology and Behavior* 47, 409-413.
- Hrupka, B. J., Gietzen, D. W. & Rogers, Q. R. (1994). Dietary limiting amino acid (LAA) concentrations alter diet selection only in LAA deficient rats. *FASEB Journal* 8, A545.
- Leung, P. M. B. & Rogers, Q. R. (1985). Effect of amino acid imbalance and deficiency on dietary choice patterns of rats. *Physiology and Behavior* 37, 747-758.
- Leung, P. M. B., Rogers, Q. R. & Harper, A. E. (1968). Effect of amino acid imbalance on dietary choice. *Journal of Nutrition* 95, 483-492.

- Naito-Hoopas, M., Gietzen, D. W., McArthur, L. H. & Rogers, Q. R. (1993). Learned preference and aversion for complete and isoleucine-devoid diet. *Physiology and Behavior* **53**, 485–494.
- Potier de Courcy, G., Durand, G., Abraham, J. & Gueguen, L. (1989). Recommendations of the feeding conditions of the laboratory animals (rats and mice). In *Science des Aliments* pp. 209–217. Paris: Centre National d'Etudes et de Recommendations sur la Nutrition et l'Alimentation.
- Richter, C. P. (1956). Salt appetite in mammals: its dependence on instinct and metabolism. In *L'instinct dans le Comportement des Animaux et de l'Homme*, pp. 577–629 [M. Autuori, editor]. Paris: Masson et Cie.
- Rodgers, W. (1967). Specificity of specific hungers. *Journal of Comparative and Physiological Psychology* **64**, 49–58.
- Rodgers, W. & Rozin, P. (1967). Novel food preferences in thiamine-deficient rats and rats recovered from vitamin deficiency. *Journal of Comparative and Physiological Psychology* **63**, 421–428.
- Rogers, Q. R. & Harper, A. E. (1970). Selection of a solution containing histidine by rats feeding on an histidine imbalanced diet. *Journal of Comparative and Physiological Psychology* **72**, 66–71.
- Rogers, Q. R. & Leung, P. M. B. (1973). The influence of amino acids of the neuroregulation of food intake. *Federation Proceedings* **32**, 1709–1719.
- Rozin, P. (1965). Specific hunger for thiamin: recovery from deficiency and thiamin preference. *Journal of Comparative and Physiological Psychology* **59**, 98–101.
- Rozin, P. (1967). Specific aversions as a component of specific hungers for thiamine. *Journal of Comparative and Physiological Psychology* **64**, 237–242.
- Rozin, P. (1968). Specific aversions and neophobia as a consequence of vitamin deficiency and/or poisoning in half wild and domestic rats. *Journal of Comparative and Physiological Psychology* **66**, 82–88.
- Rozin, P. (1976). Selection of foods by rats, humans, and other animals. In *Advances in the Study of Behaviour*, vol. 6, pp. 21–76 [J. S. Rosenblatt, R. A. Hinde, E. Shaw and C. Beer, editors]. New York: Academic Press.
- Rozin, P. & Rodgers, W. (1966). Novel-diet preferences in vitamin-deficient rats. *Journal of Comparative and Physiological Psychology* **61**, 1–4.
- Rozin, P., Wells, C. & Mayer, J. (1964). Specific hunger for thiamin: vitamin in water versus in food. *Journal of Comparative and Physiological Psychology* **57**, 78–84.
- Said, A. K., Hegsted, D. M. & Hayes, K. C. (1974). Response of adult rats to deficiencies of different essential amino acids. *British Journal of Nutrition* **31**, 47–57.
- Sanahuja, J. C. & Harper, A. E. (1962). Effect of amino acid imbalance on food intake and preference. *American Journal of Physiology*, **202**, 165–170.
- Scott, E. M. & Verney, E. (1947). Self selection of diet: the nature of appetites for B vitamins. *Journal of Nutrition* **37**, 471–480.
- Scott, E. M. & Verney, E. (1948). Self selection of diet: the appetite for thiamin. *Journal of Nutrition* **37**, 81–91.
- Sidransky, H. & Miloslav Rechcigl, J. R. (1962). Chemical pathology of acute amino acid deficiencies: comparison of morphologic and biochemical changes in young rats fed protein-free or threonine diets. *Journal of Nutrition* **78**, 269–277.
- Simson, P. C. and Booth, D. A. (1974a). The rejection of a diet which has been associated with a single administration of an histidine-free amino acid mixture. *British Journal of Nutrition* **31**, 285–297.
- Simson, P. C. & Booth, D. A. (1974b). Dietary aversion established by a deficient load: specificity to the amino acid omitted from a balanced mixture. *Pharmacology Biochemistry and Behavior* **2**, 481–485.
- Viarouge, C., Caulliez, R. & Nicolaïdis, S. (1991). Effects on metabolic and hormonal parameters of monosodium glutamate (umami taste) ingestion in the rat. *Physiology and Behavior* **49**, 1013–1018.
- Zahorik, D. M. & Maier, S.F. (1974). Preference for tastes paired with recovery from thiamin deficiency in rats. *Journal of Comparative and Physiological Psychology* **87**, 1083–1091.