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Growth and longevity of rats fed an agar-bound diet

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Summary

A conventional diet fed to rats in an agar-gel base reduced mature bodyweight by about 25% and increased longevity when compared to the same diet in pelleted form. Dry matter intake was not affected, but food utilization was considerably poorer. The use of a diet in agar gel may, therefore, provide an improved toxicological assay, especially when used in combination with flushing racks housing 2 animals per cage, where exposure of the animal technician to dust was reduced.

Purified diets in an agar-gel base have been used for more than a decade to study the carcinogenicity of various substances, including aflatoxin B_1 (Wogan & Newberne, 1967; Newberne & Wogan, 1968; Wogan, Paglialunga & Newberne, 1974). These authors claim to have demonstrated several important advantages over other types of diet for carcinogenesis or other experiments involving dietary incorporation of toxic or carcinogenic substances. They include ready acceptance by the animals and optimal growth performance. Also, because of the high water content (50%) and the consistency of the diet, spillage and wastage by the animals is minimal and it is suggested that airborne dust is entirely avoided.

Environmental contamination from a meal diet was studied by Sansone, Losikoff & Pendleton (1977) using sodium fluorescein. They demonstrated a wide spread of fluorescein contamination, both on surfaces and respirator filters, suggesting that during the conventional feeding of a test chemical personnel would be exposed and cross-contamination of animals might occur. Sansone & Fox (1977) subsequently studied the spread of fluorescein using an agar-gel diet and suggested that the spread was less.

Hence, when faced with the problem of assessing the long-term effects of feeding an inorganic fibre, with the attendant dust problem, a gel-based diet was an obvious choice. Since we had no experience of this type of diet and little or no comparative data are available in the literature, the growth and longevity data resulting from the feeding of the agar-gel bound diet to rats throughout their lifespan was compared with the data obtained when feeding the same diet in a conventional pelleted form.

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Materials and methods

Diet

The diet was supplied by Oakes (Millers) Ltd, Congleton, UK, and fed in an agar gel. The agar, 325 g (Oxoid Ltd, Basingstoke, UK), was added to 13 l water and dissolved by heating to 100°C in an autoclave for 20 min. After transferring the agar solution to a mixer, 6·5 kg of ground basic diet was incorporated. The resultant gel (33% of the ground diet being incorporated into an agar base) was poured into plastic trays, allowed to set at room temperature, and stored in a refrigerator at 4°C until required. Fresh diet was offered daily since the gel diet developed mould growth if stored in an animal room for 5–7 days. All animals received food and water ad libitum. Conventionally the diet would have been fed in a pellet (approximately 15 x 15 x 30 mm).

Animals and accommodation

Alderley Park Wistar-derived rats (equivalent to MRC Category 4) were obtained at 7–8 weeks of age from the breeding colony maintained at Imperial Chemical Industries Ltd, Pharmaceuticals Division, Alderley Park, UK. Animals fed the gel diet were housed 2 per cage in stainless-steel cages fitted with an automatic watering system. The cages were constructed of 2 mm diameter stainless-steel wire on 2 sides and floor with a 1 mm thick stainless-steel sheet back and remaining side. The solid side was included to prevent direct contact between animals in adjacent cages. The overall cage dimensions were 355 x 205 x 225 mm with a food hopper on the front of the cage, giving a floor area available to the animals of 254 x 205 mm. Troughs below the cages were manually flushed with water twice daily to remove faeces and urine.

Conventionally, animals are housed 4 per cage in mobile rat racks. The cages are constructed of 1 mm diameter galvanised wire mesh (10 x 10 mm) on 3 sides and floor, with a solid back, and measure 330 x 275 x 130 mm. They are suspended over collecting trays lined with absorbent paper. Attached to each cage is a removable food hopper

(capacity 400 g) and 2 water bottles (each with a capacity of 225 ml).

The gel diet data is based on a group of 48 male and 48 female rats. Also presented are data obtained from animals fed the diet in pelleted form. A control group (48 males and 48 females) from another experiment running concurrently in the same barrier-maintained facility, but in separate rooms, was used. This was considered to be more relevant than pooled control data since the environmental conditions would have been identical. They received the same batches of diet (pellets being milled to produce the powdered diet) and both sets of animals were drawn from the breeding population at the same time.

Observations

Clinical observations, bodyweights and food consumption were recorded regularly. Food wastage was collected for animals fed the gel diet. Consumption of gel diet was calculated on a dry weight basis by drying waste diet and a reference cube (approximately 60 g) in a vacuum oven for 24 h. Reference cubes of diet were taken from each tray of diet on the day of use. The food consumption of the pelleted diet was also calculated on a dry matter basis to make it comparable with the gel diet.

Atmospheric monitoring

The room housing the gel diet study and that housing the pelleted diet study were monitored for dust levels generally and particularly in the breathing zone of the animal technician: Static dust samplers (Type L-60; Rotheroe & Mitchell Ltd, South Ruislip, UK) sampling at 60 l/min were used to monitor the animal room environment for repeated 24 h periods over a period of 14 days. Dust levels were recorded on preweighed filters and the volume of air drawn through the samplers recorded. The dust in the breathing zone occupied by an animal technician while carrying out the normal feeding, watering and cleaning procedures each day was recorded using a personal monitor worn on a technician's lapel and attached to the same type of static dust sampler. Dust levels were measured on the same filter throughout the 14 day period so that a meaningful figure was obtained.

Results

Clinical observations

Animals fed the gel diet were in good clinical condition throughout the experiment, but appeared generally thinner than those fed the pelleted diet.

Mortalities

Animals fed gel diet showed lower levels of mortality than those fed the pelleted diet (Fig. 1), this being particularly noticeable for females where the mortality at 104 weeks was 17% for gel diet compared with 58% for the pelleted diet. A complete lifetime comparison of mortality levels cannot be made since the rats in the pelleted diet were killed at 104 weeks, but it should be noted that females fed gel diet did not reach 58% mortality until 130 weeks, although males fed either diet showed similar mortality at 104 weeks. Animals fed a pelleted diet had previously reached 80% mortality by about week 120, whereas animals fed the gel diet showed only a 50% mortality at that age.

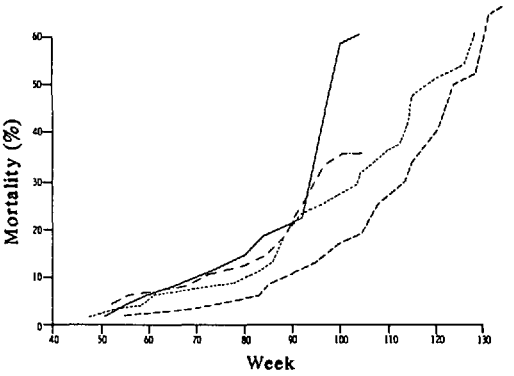

Fig. 1. Mortality of rats fed pelleted or agar-bound diet. Female rats: — pellets; – – agar. Male rats: – · – · – · – · pellets; · · · · · · agar.

Bodyweights

Mean bodyweights of both males and females fed gel diet were consistently lower than those of the animals fed the pelleted diet (Fig. 2). The difference

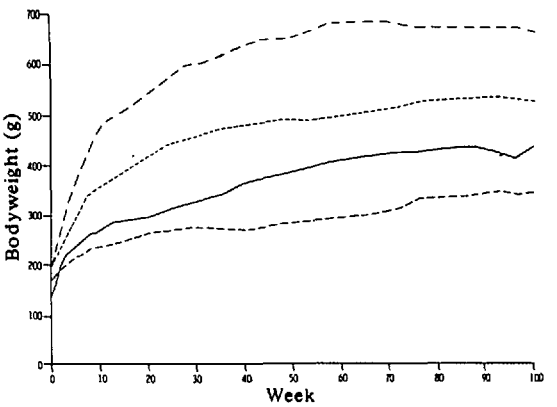

Fig. 2. Mean bodyweights of rats fed pelleted or agar-bound diet. Female rats: — pellets; – – agar. Male rats: – · – · – · – · pellets; · · · · · · agar.

appeared during the maximum growth phase and was then maintained throughout the animal's life span, being generally of the order of 25%.

Food consumption

Weeks 1–4 were selected to cover the maximum growth phase and the other weeks to show the typical food consumption during the study (Table 1). Food consumption for animals fed the gel diet was comparable with those fed pelleted diet and in fact appears to be higher during the first 20 weeks of the study.

Food conversion ratio

The food conversion ratios (amount of food eaten to gain 1 g bodyweight) are given in Table 2. The values show that the diet is less efficiently utilized when bound in an agar gel, the differences being roughly 2-fold during the maximum growth phase (weeks 1–4). At week 12 the values were more similar for the males, but from then onwards any small variation in the weekly bodyweight gain makes a large difference to the food conversion ratio, making it meaningless. However, since the bodyweights of the animals fed the gel diet were consistently below those fed the pelleted diet and food consumptions were similar, there is no evidence to suggest that the food conversion ability of either set of animals was different later in the study.

Atmospheric monitoring

There was no statistically significant difference (Student's *t* test) in the atmospheric dust concentrations between the rooms. However, there was a difference in the cumulative exposure of the animal technician, a 50% reduction in dust in the breathing zone being observed for the room housing the gel diet study compared to that housing the pelleted diet study (Table 3).

Discussion

Although agar gel has previously been used to bind purified diets, the publications reporting the use of such diets have concentrated on the carcinogenicity of individual chemicals rather than on a discussion of the growth and longevity data obtained. In addition, no references have been found which use a complete commercial diet, bound in an agar gel. It is, therefore, appropriate to discuss the performance of rats fed a diet bound in an agar gel and to compare this with that normally found when feeding the same diet in conventional pelleted form. The authors chose a control group from another experiment to provide data for pelleted diet since this was thought to be more appropriate than pooled background data for several reasons: the animals were from the same genetic population obtained at a similar time; they were fed the same batches of diet; they were housed in a similar environment. There was a difference in the numbers of animals per cage – 2 for the agar gel diet and 4 for the pelleted diet – but in another experiment animals fed pelleted diet and housed 2 or 4 per cage showed similar growth curves. In a recent study where rats were housed 2 per cage, the growth for both sexes fell in the range normally experienced when the animals are housed 4 per cage. The differences expected when housing rodents 2 or 4 per cage are, therefore, considered unlikely to account for the differences of the magnitude of those found and this agrees with the literature, where the differences were generally <10% (Wiberg, Airth & Grice, 1966; Hughes & Nowak, 1973; Chvédoff, Clarke, Irisarri, Faccini & Monro, 1980).

Table 1. Mean consumption of pelleted or agar-bound diet per rat in grams of dry matter per week

	Females		*Males*	
Week	*pellets*	*agar*	*pellets*	*agar*
1–4	107·4	149·9	153·1	176·7
20	112·5	144·2	157·5	181·0
36	115·9	116·6	145·2	159·1
52	123·6	127·2	142·7	161·4
68	125·7	139·4	144·8	155·6
84	130·6	149·3	163·3	163·1
100	125·4	169·6	167·5	189·4

Table 2. Food conversion ratio for rats fed pelleted or agar-bound diet

Amount of food eaten (g dry matter) to gain 1 g bodyweight

	Females		*Males*	
Week	*agar*	*pellets*	*agar*	*pellets*
1	10·22	3·38	5·96	2·92
2	14·46	4·89	9·34	3·30
3	14·87	6·07	8·79	4·51
4	20·00	8·61	8·71	4·94
12	42·35	19·92	30·71	32·81

Table 3. Atmospheric monitoring for dust particles (mg/m³) in rooms where pelleted or agar-bound diet was fed to rats

	Pellets	*Agar*
Personal monitor	0·93	0·45
Room monitor	mean 0·056	0·077
	sd 0·027	0·032

Age-related bodyweights were consistently about 25% lower in rats fed the diet in an agar gel compared to those fed the same diet in pelleted form. Wogan & Newberne (1967) described the growth of animals fed a purified diet bound in an agar gel as optimal, and this agrees with the findings of our work since the animals fed the agar gel diet were in good clinical condition and less obese. A lower mature bodyweight has previously been produced in the rat by restricting food intake (Nolen, 1972; Berg & Simms, 1960), by restricting caloric intake (Ross, 1972), by the use of bulk formers (Carlson & Hoelzel, 1948), or by feeding a modern maintenance diet with low dietary protein and energy levels (Clapp, 1980).

Despite the increased bulk of the agar-gel diet (binding of 67% water), there appears to be a slightly higher dry-matter intake for the animals, showing that the agar-gel fed animals compensated for the low nutrient density of the diet by eating larger volumes. Keane, Smutko, Krieger & Denton (1962) studied the effect of water addition to purified diets on growth and protein efficiency ratio. In an 18% protein diet (a similar level of dietary protein to our diet) an addition of 50% water produced a 30% reduction in weight gain, and reduced the efficiency with which dietary protein was converted to bodyweight gain.

The fact that animals fed the agar-gel diet grew slower could not be explained by restriction in energy or protein intake, since the small diluting effect of including agar (50 g/kg dry matter) in the diet was compensated for by increased food intake (g dry matter). Consequently the food utilization was less efficient (about 2-fold) despite the same amount of nutrients being available. Harmuth-Hoene & Schelenz (1980) have shown that the inclusion of 10% agar in a purified diet reduced the absorption of minerals (calcium, iron, zinc, copper, chromium and cobalt) from the gastro-intestinal tract. The volume of water added to their purified diet was likely to have been considerably less than 67%, since they state that 'the dry food ingredients were mixed with sufficient water to form a paste'. The apparent good health of the animals precludes the loss of any one micro-nutrient as no deficiency symptoms were observed, but it is probable that there was a general reduction in the availability of nutrients. Possible mechanisms for the general reduction in nutrient availability include a shortened intestinal transit time, an unspecific binding of the nutrients within the gel during its passage through the gut, or a reduction in nutrient absorption due to a change in the microbial population of the gut.

The abrupt increase in mortalities at 80–100 weeks for female rats fed pelleted diet was observed in 2 other studies carried out at a similar time and was due to a high incidence of mammary-gland and pituitary tumours. A later study on 2 animals per cage showed a marginally lower mortality – 50% at week 104 and 80% by week 118 – but even this is much higher than the mortality observed when feeding the agar-gel diet. The increased longevity associated with feeding the agar-gel diet could be of benefit in toxicological and carcinogenicity testing, as lower mature bodyweight and increased longevity have been shown to decrease or delay the onset of natural age-related changes, including tumour incidence (Berg & Simms, 1965; Ross & Bras, 1971). This may make it easier to interpret effects induced by test substances. However, it could have the disadvantage of necessitating longer studies for assessing carcinogenicity, with consequent increased costs, since the regulatory requirement necessitates feeding the test substance throughout the natural lifespan of the animal. The cost of an agar diet study will already be increased by about 15% because of the labour intensity of diet preparation and husbandry, including the daily feeding of a gel diet. In addition, the cost of the diet was increased 2½-fold by the inclusion of agar. However, diet is normally a small proportion (<4%) of the total cost of a study.

A disadvantage of gel diets is the need to handle hot agar at temperatures around 90°C, and this creates a safety hazard for the operator and needs extreme care. It also means that this method is not suitable for the inclusion of heat-labile materials into the diet. Cold gelling agents are currently being investigated by one of the authors.

In the study reported here, we used static dust samplers rather than a chemical such as fluorescein to monitor atmospheric contamination since the test substance was an inorganic fibre and hence posed a potential dust problem, although the results obtained with the personal atmospheric monitors support the finding of Sansone & Fox (1977) of a reduction in the spread of respirable contamination using gel-based diets. It should be noted that we used flushing racks with 2 rats per cage when feeding the agar-gel diets, so that it could be argued that it was the complete husbandry system rather than gel diet per se which produced the change. No differences in dust levels were found in the animal rooms, but as these were low compared with levels found in industrial districts and cities (Billings, 1974) it would have been difficult to demonstrate improvements.

It can be concluded that the combination of feeding an agar-gel diet and using flushing racks with 2 animals per cage reduces any airborne hazard to the animal technician. The effects of reduced bodyweight and increased longevity when feeding

such a diet may provide an improved toxicological assay.

Acknowledgement
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Wachstum und Lebenserwartung von Ratten, die mit einer agargebundenen Diät gefüttert wurden

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Zusammenfassung:
Wurde eine konventionelle Diät in Agar eingegossen und an Ratten verfüttert, so reduzierte sich das Erwachsenengewicht um 25% ; die Lebenserwartung im Vergleich zur selben Diät in pelletierter Form erhöhte sich. Die Trockensubstanzaufnahme war nicht verändert, aber der Futteraufwandsindex war beträchtlich erhöht. Die Verwendung einer Diät in Agargel kann eine Verbesserung bei toxikologische Versuchen bringen, vor allem wenn sie in Kombination mit der Haltung von je 2 Tieren pro Käfig in Käfiggestellen mit Wasserspülung erfolgt, bei der die Belastung der Tierpfleger durch Staub reduziert wird. (G)