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III. EFFECT OF ALUMINIUM ON GROWTH AND REPRODUCTION IN THE RAT.

THE use of rats for testing the biological importance or significance of foodstuffs and accessory factors is of comparatively recent introduction. This paper deals with the effect of aluminium in the rat when various essential constituents of the diet are varied, as well as with its effect when added to normal diets.

Other workers who have tested the effect of aluminium on rats include Myers and Mull [1928], McCollum, Rask and Becker [1928], Rose and Catherwood [1929-30] and Massatch [1930]. In all these experiments, aluminium added to the ration in amounts up to 0.1 % produced no effect when the diet was otherwise adequate. Lyman and Scott [1930] reported that no change in blood constituents (non-protein nitrogen, *etc.*) occurred on such diets. Kraft [1930] stated that the digestibility of food was not impaired by addition of aluminium. *Post mortem* examinations performed by most of these workers indicated no pathological effects, and very slight or negative absorption of aluminium.

Slight positive evidence was adduced by Daniels and Hutton [1925], who reported that rats fed on milk did not reproduce for more than two generations, but that additions of soya bean ash or the equivalent inorganic salts (aluminium, manganese, silicon and fluorine) caused satisfactory reproduction for six generations. The effect of the separate elements was not tested; but Orent and McCollum [1931] have stated that manganese is essential in reproduction.

Toxic effects due to aluminium have been reported by Schaffer *et al.* [1929] working with mice, particularly as to impairment of reproduction, histological examination of the ovary showing that this was markedly affected.

The adequacy of the diet and excessive extent of the aluminium dosage has been questioned by McCollum *et al.* [1929; see also reply by Schaffer *et al.*, 1929]. Bertrand and Serbescu [1931] have reported that the toxicity of aluminium salts injected parenterally is less than that of nickel or copper in the case of rats and rabbits.

EXPERIMENTAL.

It was originally intended to test the effect of raising several generations of rats on a diet entirely devoid of aluminium, as well as to test the effect of adding various levels of aluminium salts to the diet. Unless the former procedure is carried out, effects due to traces of aluminium cannot be excluded. A synthetic ration was employed, each component of which could be purified and tested separately, but certain of these—the vitamin-containing materials and particularly yeast—offered great difficulty. It is almost impossible to eliminate the inorganic constituents from yeast or marmite, yet to prepare vitamin B concentrates, especially at the time when these experiments began, was considered to be beyond the scope of this work. Hence the problem became one of limits—a variety of suitable constituents being tested for aluminium, and those containing the minimum quantity selected for use. Samples of these materials were ashed and examined spectrographically, and Table I gives the maximum and minimum amounts found in each constituent used over a period of 18 months.

Table I. *Maximum and minimum amounts of aluminium found in foodstuffs.*

Material	Starch	Caseinogen	Yeast	Butter	Food mixture
Aluminium in parts } Maximum	0.3	0.2	3.7	1.5	0.5
per million } Minimum	0.1	—	1.2	—	—
Lowest limit observable*	0.1	0.05	0.6	0.1	0.4

* About 1 part of aluminium in 20,000 parts of inorganic ash can be detected, so that the ash content of each substance is the limiting factor in the spectrographic determination of aluminium. The method can be made roughly quantitative [see McCollum *et al.*, 1928].

As the food mixture contained only small portions of yeast (see Table II of rations for details) the limits of aluminium content could be much higher than with the other constituents without unduly raising the total aluminium. Samples of the control ration, therefore, never contained more than 1 part of aluminium in 2 millions. Effects of amounts smaller than this, if such exist, are excluded from consideration here.

The upper limit of aluminium for experiments where this formed a part of the diet was fixed at about 0.08 %. This amount may be administered in the food in the form of phosphate (baking powder residues) or sulphate (alums) without producing cathartic effects, and is analogous to the amount ingested by the human being when living upon a mixed diet [Smith, 1928].

Exp. 1. Thirty rats weighing 50–100 g. were used, 20 receiving the synthetic diet only, the other 10 with the addition of 0.1 % aluminium as soda

alum. (The rations are set out in Table II.) They were allowed as much food as they could consume, together with water. The animals were at first placed in separate glass cages (large battery jars), with wire mesh mats covering the bottom, under which was placed a pad of several layers of filter-paper, the mouth being fitted with a glass plate with gaps for ventilation. Subsequently, the rats were placed in groups of three in the same cages, as they were unfavourably affected by isolation. In later experiments, the use of glass cages was given up, since, although they afforded an environment free from aluminium, this precaution was unnecessary owing to the presence of aluminium in the food, and in general working, glass cages were found to be fragile in use, and extravagant in space and labour. They were only used for special purposes subsequently (*e.g.* metabolic experiments, as described elsewhere [Mackenzie, 1931]).

Table II. *Diets.*

(All components expressed as percentages.)

(All components expressed as percentages.)								Aluminium*	
Diet	Yeast	Casein- ogen	Cellu- lose	Butter	Lard radio- stol	Starch	Salt mixture 185	Com- pound baking powder	Meta
(a) Complete:									
A	5	18	2	28	—	43	4	—	—
B	5	18	2	—	—	41	—	2.0†	0.094
C	5	18	2	—	—	39.6	—	3.4	0.1065
(b) Vitamin A deficient (?) or absent:									
D	6	22.5	2.5	10	—	54	5	—	—
E	6	22.5	2.5	10	—	51	5	3.0	0.094
F	6	22.5	2.5	—	10	54	5	—	—
G	6	22.5	2.5	—	10	51	5	3.0	0.089
H	6	22.5	2.5	10	—	47.2	5	6.8	0.173
(c) Vitamin B deficient (?) or absent:									
V	2	22.5	2.5	20	—	55	—	—	—
W	2	22.5	2.5	20	—	52	—	3.0	0.082
X	0	22.5	2.5	20	—	55	—	—	—
Y	0	22.5	2.5	20	—	52	—	3.0	0.082
Z 1	0	22.5	2.5	—	20	55	—	—	—
Z 2	0	22.5	2.5	—	20	52	—	3.0	0.087

* Actual aluminium content of the food as determined by analysis of samples: not necessarily corresponding to the amount of baking powder residue, which had varying composition and moisture content.

† As soda alum.

The growth curves for rats of Exp. 1 are shown graphically in Fig. 1. The method adopted is to show the average growth for the groups of one sex together with the individual highest and lowest growth curve. It is considered that this gives the fairest computation of the results obtained. Only the growth curves of females are shown in this case, the males showing a general correspondence but about 20 % greater individual weight.

When maximum weight had been reached, and breeding tests were satisfactory, as shown by the production of viable young by each female (see

Table III, under appropriate experiment) a proportion of the animals were killed and the organs analysed for aluminium, using the colorimetric methods of Myers and Morrison [1928]. Owing to the small amount of material obtained from individual rats, it was necessary to carry out these analyses on groups of five or more animals. The results are shown in Table IV.

Fig. 1, Exp. 1. Upper curve—heaviest female; lower curve—lightest female; middle curve—average for all females in group.

Table III. *Fertility of animals used in experiments.*

Note. The animals were placed in groups of three, one male and two females as far as possible. If no progeny resulted, the male was considered sterile and a male from another group employed. If only one female produced young, the other was tested with another male before being pronounced sterile. The production of viable young was thus the criterion. Excess males were tested when circumstances allowed.

Exp.	Sex	Control group				Aluminium group			
		Total number	Fertile	Sterile	Not tested*	Total number	Fertile	Sterile	Not tested*
1	♂	4	4	0	—	3	3	0	—
	♀	16	12	0	4	7	5	0	2
2	♂	7	6	1	—	8	5	2	1
	♀	8	6	0	2	7	7	0	—
1 A (first generation)	♂	7	5	0	2	8	5	0	3
	♀	13	10	1	2	10	9	1	—
1 A (second generation)	♂	6	4	0	2	8	5	0	3
	♀	9	9	0	—	17	10	0	7
1 A (third generation)	♂	7	6	0	1	11	6	0	5
	♀	12	8	0	4	9	9	0	—

* Including premature deaths.

Exp. 2. This was a repetition of Exp. 1, but the rats were placed in groups of three in glass cages, and the aluminium was supplied in the form of a decomposed baking powder, containing about 3% of aluminium. To decompose it, this was heated on a water-bath with half its weight of water, and dried at 100°. The product was added to the ration at a level of 3–3.5%, replacing an equal weight of starch, but as 50% of the baking powder itself consists of starch, the variation in this item in the diet was insignificant (see ration C in Table II). The experiment lasted 10 months, and the growth

curves, breeding results and analyses appear in the respective Tables and Figures.

Fig. 2, Exp. 2. Upper curve—heaviest female; lower curve—lightest female; middle curve—average for all females in group.

Exp. 1A et seq. Effect of aluminium upon subsequent generations. Progeny of rats employed in Exp. 1, both control and aluminium-fed animals, were

Fig. 3, Exp. 1A. Upper curve—average for all males in group, and number of males. Lower curve—average for all females in group, and number.

used to determine if aluminium at a high or low level could exercise any depressant effect upon growth or fecundity in subsequent generations. The rations used were diets A and C, and three generations were successfully raised. In each generation, about twenty of the young were selected from all

the litters available, and the remainder discarded. In order to shorten the time covered by these experiments, breeding was allowed to commence at the earliest possible age, the sexes not being separated, and the first litters were used as representatives of the next generation. This may explain why the growth curves in all generations are on the low side in both groups. Considerable mortality occurred in the second generation owing to extensive and unavoidable changes of temperature in the cage room during the progress of this experiment. In the case of the aluminised groups, five died, and others from the same generation and on the same diet, which had been set aside for metabolic experiments, were brought in to replace them. In these experiments the rats were placed in the usual type of metal cages in groups of five, the diet being supplied *ad lib.* and unlimited breeding being allowed. The results are shown in growth curves, breeding Table and analysis.

As no perceptible effect due to aluminium could be observed when the diet was sufficient for normal requirements, it was decided to make tests under less favourable conditions, *viz.* when the vitamin factors in the diet were reduced to the lowest level sufficient for maintenance, or below. It seemed possible that any differential effect due to aluminium would be more marked under these conditions.

Exp. 3A. Twenty rats were divided into two groups receiving rations D (control) and E (aluminised) respectively. The rats weighed 40–60 g. at the commencement of the experiment. The fat content and vitamin A, supplied

Fig. 4, Exp. 3 A. Low vitamin A (males).

in the form of butter, were thus reduced to one quarter of the normal. Growth did not vary significantly between the groups and was little below normal generally, though the mortality was higher. After 11 months, 7 rats remained in the control groups and 5 in the aluminium group, they were killed and used for analysis. Growth curves and analytical results are shown in Fig. 4 and Table IV.

Exp. 3B. Effect of avitaminosis-A. Twenty rats, 10 receiving diet F (control) and 10 receiving diet G (aluminised), initial weight about 100 g.,

were employed. The ration contained lard instead of butter, thus excluding vitamin A. The growth curves fell off sharply after 6 or 8 weeks, and no animal survived 4 months after the experiment began, yet no convincing evidence was obtained that aluminium had any accelerating or retarding effect upon the morbidity. In each group individual growth curves of five individuals, including the extremes (earliest and latest deaths) are shown (Fig. 5).

Fig. 5, Exp. 3B. No vitamin A (animals weighed 100 g. to commence).

Exp. 3C. Effect of feeding high levels of aluminium. Ten rats, being surplus offspring of rats of Exp. 1, were placed on diet G (containing 1.7 % of aluminium as baking powder residue, *i.e.* aluminium phosphate), and their growth was compared with that of 5 rats placed on diet C, similar in all respects except for the omission of the baking powder. Growth was distinctly below normal, but it was noticeable that the rats did not eat the diet readily, and the consumption per head was much less than in the control group (about 9 g. against 13 g.). The ration had a pronounced saline flavour and apparently induced thirst. Analysis of the animals after 6 months on the diet showed no noticeable storage of aluminium in the organs.

Fig. 6, Exp. 3C. High aluminium diet (highest, average and lowest growths in group).

Exp. 3D. Effect of limiting the vitamin B complex. A group of 40 rats, about 40–60 g. in weight, was divided into 4 groups of 10, receiving rations

V, W, X and Y (which contained little or no yeast, with and without additions of aluminium). They were caged in groups of five, and coprophagy was prevented as usual. A number of the animals exhibited spasmodic inco-ordination in the early stages, and this was treated by the administration of 10–20 cc. of milk to the affected animal. This produced rapid recovery without supplying excessive amounts of vitamin B. Very few of the animals died during the first 2 months, and the growth curves showed steady if small increases—indicating that butter is a sufficiently rich source of vitamin B when fed at a level of 18 % to maintain growth in the early stages of life. By the sixth month extensive mortality commenced in the control group, while the aluminium group was very little affected. Under these conditions the baking powder addition may be suspected of some specific sparing action or contributory vitamin. The point was not entirely solved by the next experiment.

Fig. 7, Exp. 3D. Showing highest, mean and lowest growth curves of the groups.

Exp. 3E. Effect of aluminium on avitaminosis-B. Twenty rats were divided into 2 groups of 10, receiving diets similar to X and Y, where lard replaced the butter. Radiostol was also added. The animals were given milk in the early stages whenever inco-ordination was observed, thus effecting a temporary cure, and the experiment was on parallel lines with Exp. 3D. The mortality, as would be expected on a diet entirely deficient in vitamins A and B,

Fig. 8, Exp. 3E. Average weight of animals—50 g. at start; † = death.

was high. In 2 months 7 animals died. The milk supplement was then discontinued, but 8 animals survived after 3 months, and were apparently resistant to avitaminosis-B. After 4 months typical symptoms of avitaminosis-A set in, and only 2 animals (both controls) survived to the fifth month. These rats had shown unusual growth and vigour throughout, and apart from the exceptions, the 2 groups showed no significant difference [see growth curves of Fig. 8, Exp. 3 E].

DISCUSSION.

It is probable that the environment was responsible for the fact that the growth curves in Exps. 1 and 2 were not up to normal, but they showed little difference as between groups on control and aluminised diets. The fertility of rats in these experiments and in the subsequent generations raised from rats of Exp. 1 also show no differential effect. Exp. 3 B, in which high levels of aluminium were fed and some stunting of growth resulted, has some resemblance to the effects obtained by Schaffer *et al.* [1928]. In this case it was notable that the difference in the amount of food consumed would account for the poor growth, the rats taking very little more of it than would suffice for maintenance. To obtain clear-cut results it would be desirable (a) to feed a control group with the control diet at the same level as that at which the experimental rats would consume their ration, and compare the growth curves; (b) to feed a form of aluminium which is tasteless and non-cathartic, pure aluminium phosphate would probably be suitable, so that the diet would be more freely consumed.

Under present circumstances, the question as to whether the stunting effect is due to a specific action of aluminium, or a general lack of balance in the diet, must be considered an open one. Exp. 3 D, in which an apparent lengthening of life resulted from the aluminium addition, may be considered to be due to a specific action of aluminium, but the other components of the baking powder—starch and albumin—must not be overlooked as a possible source of vitamin B. On the whole, the avitaminosis experiments give no reason to suppose that aluminium has any action one way or the other.

The analytical results suggest that some absorption of aluminium by the liver occurs on aluminised diets. The amounts do not seem to increase cumulatively with the period of feeding, and are quite comparable with the amount found in control animals [see also Tourtellotte and Rask, 1931].

CONCLUSIONS.

- (1) Aluminium additions to the diet have no perceptible influence
 - (i) when the diet is adequate for growth and reproduction in the rat;
 - (ii) when the diet is insufficient for growth or maintenance;
 - (iii) when fed to several successive generations of rats.
- (2) Aluminium absorption from such diets appears to be exceedingly small.

IV. THE OCCURRENCE OF ALUMINIUM IN THE THYROID.

It has been reported by Underhill *et al.* [1929] that aluminium tends to accumulate in the thyroids of dogs when the animals are on an aluminium diet, amounts up to 0.07 mg. having been found in a single thyroid. Recently Tourtellotte and Rask [1931] have questioned these results. Since 1929, as occasions have offered, samples of thyroid have been secured from normal, aluminium-fed and iodine-fed animals in this Institute, in an endeavour to determine whether aluminium is present in the thyroid, and if it can be connected in any way with iodine metabolism. Iodine and aluminium have been determined in each sample (except in two cases where the total amount of tissue would not suffice for aluminium assay, in which case iodine alone was determined). The aluminium content was determined by the colorimetric method using aurintricarboxylic acid [Myers and Morrison, 1928]; the iodine by the method of Leitch and Henderson [1926]. I desire to thank Miss Stuart of this Institute for the iodine determinations referred to below.

(a) *Iodine and aluminium in the thyroids of pigs employed for
aluminium experiments* [see Mackenzie, 1931].

Pig No.	Diet	Weight of thyroid g.	Iodine in thyroid g./100 g.	Aluminium in thyroid mg./100 g.
2474	Al less than 2 parts per million	4.81	0.155	0.04
2477		4.11	0.165	0.05
2480		2.6 (part)	0.189	0.9
2475	Above diet <i>plus</i> 0.1 % aluminium	5.48	0.193	2.63*
2479		4.51	0.202	—†
2488		8.39	0.241	0.02

* Final product showed iodine coloration—result doubtful.

† Results below 0.02 are not reliable, and are left blank.

(b) *Iodine and aluminium in the thyroids of pigs employed for
iodine experiments.*

Groups of 5 pigs received (I) control ration adequate for normal growth, (II) same diet + potassium iodide, (III) same diet with ultra-violet irradiation, (IV) same diet, with potassium iodide and ultra-violet irradiation.

Group of 5 pigs receiving	Total weight thyroids g.	Iodine in thyroids g./100 g.	Aluminium in thyroid mg./100 g.
Normal diet	18.2	0.118	0.02
„ + KI	29.4	0.238	—
„ + U.V.I.	22.7	0.202	0.06
„ + KI + U.V.I.	34.1	0.097	0.03

Note. The thyroid material had been dissolved in dilute alcoholic potassium hydroxide for iodine determination, and stored in glass bottles. It was anticipated that aluminium might be present owing to the action of the potash on glass, which usually contains aluminium. But the results show that the aluminium content, from whatever source derived, is extremely small.

(c) Other samples of thyroid from pigs.

Pig	Weight of thyroid g.	Aluminium mg./100 g.
Normal, 6 months old	3.8	0.12
Depancreatized 2 months previously ...	2.1	0.07
Depancreatized 1 day previously ...	4.7	0.05

(d) Iodine in thyroids of rats receiving aluminised diets.

The thyroid in the rat is too small to be analysed for aluminium by any of the methods now in use. It was considered advisable to find the iodine content of some thyroids in the case of rats used for aluminium experiments, as described above, to see if any correlation might exist. The thyroids of rats in Exps. 1 and 2 were analysed by Miss Stuart, and the results follow:

Exp. No.	No. of rats examined	Average weight of thyroid g.	Iodine content of thyroid g./100 g.
1 (control aluminised)	10	0.017	0.042
	5	0.015	0.061
2 (control aluminised)	6	0.016	0.027
	7	0.019	0.026

These results indicate that

- (i) aluminium occurs at only a very low level in the thyroid;
- (ii) there is no correlation between aluminium and iodine in normal metabolism.

V. INTESTINAL ABSORPTION OF ALUMINIUM IN THE RABBIT.

The presence of small quantities of aluminium in the normal diet of men and animals [Smith, 1928], and the presence of aluminium in very minute quantities in the tissues [Myers and Mull, 1928], are both well established facts which have not been satisfactorily correlated. It is established that certain aluminium compounds, when present in the food, are converted into a soluble form by the action of the gastric juice [Myers and Killian, 1928] and it seems reasonable to suppose that in the upper part, at least, of the small intestine, absorption of the aluminium ions may be expected to occur as is the case with calcium, sodium and potassium, though in the lower parts of the intestine aluminium appears to be precipitated [Massatch, 1930]. The absorbed aluminium might be conveyed by the portal blood to the various organs, notably to the liver, and it has been observed that the aluminium content of the liver is greater in aluminium-fed animals than in controls. Though the amounts are in all cases very small [Underhill *et al.*, 1929] the increase is definite, and can hardly be explained on any other ground. But the presence of detectable amounts of aluminium in the blood after ingestion of aluminium compounds has rarely been observed, the findings of all workers being negative except those of Underhill *et al.* [1929]. These workers, using an exceptionally sensitive method for the detection of aluminium, found that aluminium was sometimes

absent from the blood of normal animals, and even when present the initial amount was not always increased by aluminium ingestion, while in some cases a fall was observed.

Myers and Morrison [1928] injected a known amount of an aluminium compound, usually the sulphate or chloride, into a washed intestinal loop in dogs, and found it possible to recover the aluminium, within the limits of experimental error, after a period of 1 to 4 hours. They pointed out, however, that the aluminium was recovered as a precipitate on the mucosa, and it seems certain that the normal reaction of the intestine would convert most aluminium salts to the hydroxide, which is colloidal and probably non-absorbable.

In the present experiments, a known amount of aluminium in the form of a solution of aluminium tartrate was injected into the ligatured intestine of a rabbit (which had received an aluminium-free diet for one week previous, and had fasted for 24 hours before the experiment) by the method due to Magee and Macleod [1928]. The rabbit, anaesthetised with urethane and ether, was opened up to expose the viscera, and the intestine tied off at the oesophagus, the ileal extremity being cut and sutured to a discharge tube. A small cannula was inserted below the ligature, and 10 cc. of aluminium tartrate solution (1 mg. Al per cc.) at approximately p_H 8 were injected. After an interval varying from 15 to 30 minutes, about 20 cc. of blood were drawn from the portal vein by hypodermic syringe; a second sample was drawn after a further period of 10 to 15 minutes. The animal was then bled to death through a cannula in the carotid artery, and subsequently the liver and entire small intestine were dissected out. These samples of blood and tissue were ashed in silica basins and the aluminium content was determined by the colorimetric method. In

Table IV. *Aluminium found in the blood of rabbits after injection of aluminium tartrate into intestine.*

Exp. No. ...	1	2	3	4	5	6	7	Control	
Aluminium injected into intestine (mg.)	11.05	10.8	10.4	11.05	11.05	10.3	11.05	0.0	0.0
Aluminium found (mg./100 g.):									
(a) in portal blood									
1st sample	0.017 (20)	0.047 (30)	0.000 (15)	0.016 (15)	0.000 (15)	0.000 (25)	0.000 (25)	0.000	0.016
2nd sample	0.049 (35)	0.000 (45)	0.021 (45)	*	0.000 (25)	0.000 (35)	0.000 (40)	—	—
(b) in systemic blood	0.000 (40)	0.000 (50)	—†	—	5.60†	0.000 (40)	0.083 (45)	0.000	0.000
(c) in the liver	0.56	0.86	—	0.42	0.79	0.55	1.15	1.12	0.70
Aluminium found in intestine (mg.)	1.48	8.82	9.04	11.3	9.6	7.73	9.6	0.67	0.57
Percentage recovered from intestine	13.5	81.7	86.9	102.2	87.7	75.1	87.7	—	—
Average percentage recovered in six experiments ...									86.8

The figures in brackets following the blood figures are the times (mins.) from the initial injection at which the blood sample was taken.

* This animal died 25 minutes from commencement.

† This animal died 45 minutes from commencement.

‡ This animal died 30 minutes from commencement. The systemic blood sample was obtained from the body cavity, and was possibly contaminated by the liquid injected into the intestine—the actual amount of aluminium present in 10.7 g. of blood was 0.60 mg.

addition to 7 animals thus used, 2 others were used as controls, 10 cc. of plain saline being injected into the intestine, the other operations being as before. The results are shown in Table IV.

DISCUSSION.

The average recovery in 6 experiments varied from 75.1 % to 102.2 % with a mean value of 86.8 %. The first experiment showed an extremely low recovery of aluminium from the intestine, possibly due to leakage at the site of injection. It stands alone and has been ignored in computing the average. In the 2 control animals, the aluminium level in the intestine was about 5 % of that found in the experimental animals, showing that 7 days on an aluminium-free diet was not sufficient to remove all traces of aluminium from the intestine. Hence it appears probable that the average recovery of aluminium injected is only about 82 %.

If the aluminium content of the portal blood is compared with the amount present in the intestine, it does not seem probable that aluminium is readily absorbed by the intestine. The amount circulating in the blood at any time is extremely small, but it is undeniable that aluminium may be, and frequently is, conveyed in the blood-stream.

SUMMARY.

Injection of aluminium salts into the intestine of rabbits does not cause any appreciable absorption of aluminium into the blood-stream. The aluminium injected can be largely recovered by analysis of the intestine.

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