

XXXVII. THE RELATION OF A FAT-FREE DIET TO THE SCALY TAIL CONDITION IN RATS DESCRIBED BY BURR AND BURR.

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EXPERIMENTS made before the discovery of the fat-soluble vitamins with the object of determining the indispensability or otherwise of fat in the diet have little value. No effort was made to supply the fat-soluble vitamins and failures which were really due to a deficiency of them were attributed to the lack of fat as fat.

As early as 1918 Hindhede [1918] concluded from prolonged dietary experiments on young men that fat was not necessary if a daily ration of fruit and vegetables, sufficient to supply the fat-soluble vitamins, was consumed.

Animal experiments in which the distinction was drawn between fat as fat and fat as the vehicle for the fat-soluble vitamins were first made by Osborne and Mendel [1920-21] and by Drummond and Coward [1921]. In the observations of Osborne and Mendel the diets were not completely devoid of fat, they were only relatively very low and fat-soluble vitamins were supplied as dried alfalfa. The rats thrived and grew well and the authors concluded that "if true fats are essential for nutrition during growth the minimum necessary must be exceedingly small." In Drummond and Coward's experiment the fat in the diet was reduced to a trace and fat-soluble vitamins were supplied as the unsaponifiable fraction of fish-liver oil. The rats grew well on the diet and reproduced, and the conclusion of Hindhede and of Osborne and Mendel was completely borne out, that fats, while being a convenient form of energy-bearing food, are only indispensable as carriers of the fat-soluble vitamins; when a supply of these is otherwise assured, fats can be omitted from the dietary.

For some years this view remained unchallenged, until Evans and Burr [1928] and Burr and Burr [1929] announced a new dietary deficiency in fat-free diets, which was relieved by the administration of fatty acids. The rats on the fat-deficient diet grew sub-normally and developed a scaliness and annulation of the tail, which might become necrosed, so that 1-3 cm. of the tip might be lost; the skin of the feet and also of the whole body ultimately became affected and the animals finally declined and died.

McAmis, Anderson and Mendel [1929] describe some experiments in which

rats on fat-free diets grew and throve less well than those on diets containing fat, though the fat-soluble vitamins were supplied as the unsaponifiable fraction of cod-liver oil. One case is described where the tail of the rat became dark and gangrenous, but a condition as severe as that described by Burr and Burr was not observed.

The condition of "scaly tail" which occurred among the rats used by the present writers in the experiment described in the preceding paper [1931] appears to be identical with that described by Burr and Burr, but as has already been stated, it could not be referred to the lack of fat in the dietary, since it occurred also among rats receiving abundant fat. The factors to which it might be attributed appeared to be rather the type of caseinogen, the source of B vitamins and the type of cage used, with open wire grids to prevent coprophagy. The condition has also been observed in the Lister Institute by Chick, by Roscoe and by Aykroyd (personal communications) on a variety of diets but always when using cages with coarse wire flooring, on diets containing specially purified caseinogen and usually with yeast derivatives and yeast fractions, rather than with whole yeast as the source of B vitamins. Experience in this Institute is not incompatible with the view that modification of the supply and source of yeast vitamins, under which heading the use of anti-coprophagy cages is included, is the responsible factor in the production of scaly tail.

Analysis of the experimental results of the other workers already cited is not inconsistent with such an interpretation. The early experiments of Drummond and Coward and of Osborne and Mendel, in which the rats throve on a fat-free diet, would probably have been carried out in cages with solid bottoms as was usual at that date. Evans and Burr made a first series of experiments in which cages with solid bottoms were used, while in all the subsequent ones the rats were housed on wire grids. It is remarkable that while in each experiment rats receiving fat grew better than those which did not, yet those which received fat and were caged on wire grids grew less well than ones which did not receive fat, but were housed in cages with solid bottoms. Less pure caseinogen seems also to have been used in the experiment where the cages had solid bottoms. The B vitamins were supplied as 0.7 g. daily of Fleischman yeast. No symptoms are described as having occurred in this experiment.

In the experiment of McAmis, Anderson and Mendel the conditions were somewhat similar; the experiments were carried out using, partly, cages "consisting largely of glass" and, partly, cages with floors of open wire mesh. Harris yeast concentrate was used as a source of the B vitamins and later it was supplemented with 400 mg. of yeast daily. Inferior growth was shown by the rats on the fat-free diet and in one instance a tail lesion was observed, but the experiments are not well described and it is clear from the account of the autopsies that the rats on the fat-free diet, which received their fat-soluble vitamins as unsaponifiable fraction of cod-liver oil, were partly de-

ficient in vitamin A. It is not possible to obtain much evidence from an analysis of these experiments.

In the work of Burr and Burr the rats were housed throughout on coarse wire grids (two meshes to the inch); they received a diet which contained no fat, specially purified caseinogen and ether-extracted yeast. In these conditions the symptoms already described developed severely and could be cured by 10 drops of lard or 1.0 g. of liver daily, but not by 0.7 g. of whole yeast daily. The conditions here were more drastic and the syndrome developed fully, but the cure with 10 drops of lard and the failure with 0.7 g. whole yeast do not appear to support a theory of some deficiency in the yeast vitamin complex. It might however be that in the drastic conditions of the experiment 0.7 g. of dried yeast did not supply enough of the factor, after the condition had developed severely. Aykroyd (personal communication) also only secured slow improvement of the condition when established to a severe degree, with 1.0 g. of dried yeast but a cure was brought about with a daily dose of 1.0 g. dried liver. The failure with 0.7 g. of dried yeast definitely puts any question of a deficiency of vitamin B₂ out of court.

Some work of Evans and Lepkovsky [1929] however suggests in a most interesting manner that the curative action of fat for scaly tail may be exercised by making good some deficiency in the yeast vitamin complex. They show that fat in the diet can have the effect of reducing the amount of B vitamin which the rat needs and conclude that the more fat there is in the diet up to a point the less antineuritic vitamin B₁ is needed. They do not consider that this sparing action of fat is extended to vitamin B₂, but a careful examination of their charts suggests that it is, though less effectively than in the case of vitamin B₁. If fat, therefore, can have a sparing action on the B vitamin complex, an explanation is offered of Burr and Burr's observations, which is also compatible with an ascription of the deficiency which produces scaly tail to lack of some factor within the yeast vitamin complex. Evans and Lepkovsky go no further than conjecture with regard to the mechanism by which the sparing action is produced; they suggest possible alterations in the rat's intestinal flora. The present writers would tentatively suggest, though they have no evidence in support of their suggestion, that the addition of certain fats might operate by altering the consistency of the faeces, rendering them more pasty and less liable to fall through the wire grids and thereby promoting coprophagy. At any rate an examination of the literature and a comparison of the results with the writers' own experience made it seem worth while to test further the hypothesis that the aetiology of the scaly tail syndrome lies in variation of the conditions governing the supply of the yeast vitamin complex.

EXPERIMENTAL.

The object aimed at was to compare the condition of rats which received diets containing fat or no fat, and were kept on wire screens and on solid bottoms respectively.

It was decided to use the commercial yeast extract marmite as the source of B vitamins.

Twelve rats were used which were derived from four different litters, so that it was not possible to balance four experimental groups perfectly in this respect.

For the first 50-60 days of experiment all the rats received the following diet:

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| Caseinogen (British Drug Houses, fat- and vitamin-free) ... | 20 |
| Rice starch | 72 |
| Marmite yeast extract | 4 |
| Salt mixture (McCollum 185) | 4 |

Vitamin D was given separately as 0.004 mg. of irradiated ergosterol in ethyl oleate daily, but no vitamin A was given in this period of the experiment as the animals were originally intended for another purpose.

The rats were kept in cages with a floor of such finely perforated zinc that, so far as coprophagy was concerned, it amounted to a solid floor.

About the 50th-60th day the animals showed signs of failing from lack of vitamin B₁; marmite was therefore increased to 15 % of the diet. At the same time fat was introduced into the diet of six rats as 10 % of hardened arachis oil in the place of 10 % of starch, while the remaining six rats remained on the same diet devoid of fat. Of the six rats receiving fat in the diet, three were placed in cages with coarse screens (9 to the square inch) and three remained on the same floors as before: the six on a diet without fat were similarly divided. The irradiated ergosterol from this point also was administered in solution in liquid paraffin instead of in ethyl oleate. Experiments with carotene had shown how quickly it is oxidised in ethyl oleate solution, and it was feared that vitamin D might suffer oxidation in the same way.

For the next 40 days, for the purpose of another observation, the six rats on fine screens received their vitamin A as 0.006 mg. of carotene in liquid paraffin daily, while those on coarse wires received it as 0.04 g. of fresh green cabbage daily. At the end of the 40 days, *i.e.* about the 100th day of experiment, all the rats received their vitamin A as cabbage. At the same time, since any symptoms of scaly tail so far shown were very slight, the marmite was reduced once more to 8 %; it was deemed that the supply of vitamins B₁ and B₂ would be again endangered if it were reduced any lower.

About the 180th day as the rats on the fine wires showed very little sign of the disease coarse screens were substituted.

About the 200th day, when it was clear that fat in the diet was making no difference, vitamins A and D were administered more conveniently as 5 drops of cod-liver oil daily, instead of as cabbage and irradiated ergosterol.

The total duration of the experiment was about 290 days.

On the regime above described, no rat developed a severe condition of scaly tail but all showed a recognisable degree of it, except one which died

on the 126th day. At autopsy it was found to have a cystitis, with stones in the bladder; the vitamin A supply was therefore probably insufficient for it. The growth of the animals is not considered in the results; the supply of vitamins B₁ and B₂ was insufficient for good growth over the major part of the experiment, so that the growth performances have no significance. The behaviour of the groups was as follows.

Group 1. Diet + fat. Coarse screens. Symptoms developed early and comparatively severely, with a well-marked black necrotic tip to the tail in one case, moderately in the second case and slightly in the third. The most severe case showed slow improvement when treated with 0.2 g. dried yeast for 72 days and became cured, so that even the black tip to the tail disappeared, when treated with 0.4 g. daily for 41 days. The second case improved very slowly on 0.1 and 0.2 g. daily of dried yeast, but was almost cured when the experiment was ended after it had been for 21 days on 0.4 g. The third showed little improvement when treated with 0.2 and 0.4 g. of autoclaved (120° for 5 hours) dried yeast daily but was improved after 27 days on 0.4 g. of dried unheated yeast.

Group 2. Diet - fat. Coarse screens. All the three rats developed well-marked scaliness and two showed very small necrotic black tips to their tails, one of which made its appearance while the rat was under treatment with autoclaved yeast. Treatment with 0.4 or 0.5 g. of autoclaved yeast brought about no improvement, but when the dose was changed to one of 0.4 g. of yeast, not autoclaved, over a period of 20 days, two of the rats showed improvement and one was cured.

Group 3. Diet + fat. Fine screens. Symptoms developed very slightly while the rats were on the fine screens but increased after coarse ones were substituted. One rat died without symptoms before that date was reached. The two survivors were treated with 0.2 and 0.5 g. autoclaved yeast with no benefit; a black tip to the tail was developed in one case after the treatment had begun. Improvement took place when the rats were treated with 0.4 g. dried yeast for 12 and 27 days respectively.

Group 4. Diet - fat. Fine screens. The tail of one rat remained normal up to the removal of the fine screens; the other two showed slight but slowly increasing symptoms. After the substitution of coarse screens the degree of scaliness increased and two rats developed black tips to their tails, one of them whilst under treatment with autoclaved yeast. No improvement took place with doses of 0.2, 0.4 or 0.5 g. of autoclaved yeast. With a daily dose of 0.4 g. of dried yeast improvement took place after 10, 17 and 18 days respectively.

DISCUSSION.

The condition was not developed severely enough to give very sharp results. On 15 % of marmite in the diet, it scarcely developed at all; it was only after the marmite was reduced again to 8 % that the condition became definite. Symptoms occurred approximately equally in the groups with and without

fat, though they were perhaps slightly more severe in the groups without fat. Four of the six rats without fat developed black tips to their tails, while only two did so in the two groups which received fat. On the other hand the most severe case, with the largest black necrotic area on the tail, was that of a rat which received fat in the diet.

The symptoms developed slightly or not at all when the rats were housed on fine screens and became more marked when coarse ones were substituted.

The condition was unaffected or even grew worse while the animals were being treated with 0.2–0.5 g. daily of autoclaved yeast. Dried yeast in a daily dose of 0.4 g. produced improvement, and the most severe case, on which treatment was begun early, was completely cured.

The caseinogen and the coarse wire grids were the same as were used in the sterol experiment, where the symptoms were more marked; the source of yeast vitamins was however different and one is inclined to suggest that in marmite the vitamin factors are not sufficiently unbalanced for the production of scaly tail. Even 0.4 g. of yeast only slowly cured the condition when it was very mild; it is easy to imagine that considerably larger amounts might fail to cure a very much more drastic deficiency.

The results of this experiment lend some support to the hypothesis that the production of the scaly tail condition is influenced by factors which influence the supply of the yeast vitamin complex. In certain circumstances the presence or absence of fat in the diet appears to be one of these factors, but in the conditions of the present experiment and using hardened arachis oil as the form of fat, it was not appreciably operative. Burr and Burr used lard, and Evans and Burr lard, butter-fat, corn oil and coconut oil but no hardened fat, but Evans and Lepkovsky found that the sparing action of fat was also exercised by hardened cottonseed oil, so that it seems improbable that the virtue is lost in the process of hardening. The failure of fat to exercise its protective action against the development of scaly tail in the writers' experiments therefore remains unexplained, but it seems to exclude the hypothesis that scaly tail is the direct result of a pure fat deficiency.

SUMMARY.

1. A scaly tail condition, considered to be the same as that described by Burr and Burr and ascribed by them to lack of fat in the diet, was developed in the course of experiments in which the rats received diets with and without fat.

2. The condition was developed when the rats were kept on coarse wire grids, with highly purified caseinogen and usually when extracts of yeast, rather than whole yeast, were used as the source of B vitamins.

3. A comparison of the behaviour of rats housed on coarse and fine grids showed that the development of the condition was favoured by the use of the former.

4. The condition, when developed in a mild degree, could be cured by

the addition of whole dried yeast. Autoclaved yeast conferred no benefit, showing that the condition could not be due to an insufficiency of vitamin B₂.

5. The literature is discussed and it is suggested that the scaly tail condition may be correlated with some deficiency in the yeast vitamin complex and is influenced by the same factors as influence the action of the B vitamins, *i.e.* opportunities for coprophagy and the amount of fat in the diet.

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