LX. A COMPARISON IN FIVE TYPES OF ANIMALS OF THE EFFECTS OF DIETARY EGG WHITE AND OF A SPECIFIC FACTOR GIVEN ORALLY OR PARENTERALLY[1]

BY JANE GERMER LEASE, HELEN TRACY PARSONS AND EUNICE KELLY

From the Home Economics Department, University of Wisconsin, Madison, U.S.A.

(*Received 29 January 1937*)

IN characterizing a given nutritive disorder it is of distinct advantage to produce it in more than one type of animal. Especially is this true if the disorder is complex in nature such as the one under consideration due to dietary egg white wherein there is an interrelationship between an injury and a relative deficiency [Parsons *et al.* 1937].

The manifestations of injury in the rat due to dietary egg white, as previously described [Parsons & Kelly, 1933; Lease *et al.* 1936], include redness, soreness and bareness of lips and paws, redness and swelling of eyelids, scurf, pigmentation, extensive loss of hair and a marked nerve disorder. The symptoms occurring in the chick as described by Lease & Parsons [1934] include swelling and reddening of eyelids, loss of feathers, cracking and bleeding of the skin of the feet and the appearance of a bloody scab at the corners of and under the mandible. The symptoms in both types of animals may readily be cured by foodstuffs containing the potent factor.

The purpose of the present paper is to record the effects produced on three other types of animals and to make some comparisons among them.

Experiments with the guinea-pig

The first experiments, employing slight modifications of the standard basal ration containing 66 % of dried egg white used in experiments with rats, resulted in the steady decline of the guinea-pigs, terminating in prostration, convulsions and death in 5–10 days. Haematuria was present in some cases and haemorrhage of the digestive tract in others. A preliminary dose of 3 g. of cooked pork liver given the day before the egg white ration was begun, a procedure which successfully protected the rat, did not delay death in the guinea-pig. A number of foodstuffs curative of symptoms in the rat were tested in the ration without success. It was found necessary to introduce the egg white ration gradually; when the egg white was raised step-wise to 50 % of the ration the guinea-pig tolerated this level fairly well.

The animals usually became inactive and hunched, and the hair dull, unkempt, matted and sticky with the egg white ration on lips and toes, but no bald or sore areas developed except, in a few cases, a transient bareness and redness of the lower lip and the toes, which was rather mild in character. One guinea-pig had scaly skin on the soles of the feet. It was uncertain whether or not the bareness was attributable to a pulling off of the hard lumps of food embedded in the hair.

[1] This work was supported in part by a grant from the Alumni Research Foundation. Published with the permission of the Director of the Agricultural Experiment Station.

When 20 % of dried cooked pork kidney was incorporated in the ration, the general condition of the animals was noticeably superior to that of the others; they were more alert and active and the hair was glossier and smoother. However, transient redness and bareness of the lip was observed in this group also, although not to the same extent as in the other.

A new lot of guinea-pigs was established on the following ration: egg white 30 %, alfalfa 5 %, irradiated rice polishings 10 %, salt mixture 2·5 and later 4 %, butter fat 8 %, starch and sucrose in equal amounts to make 100 % and daily addition of 5 g. of cabbage or of cabbage and carrots. On this diet the possibility of injurious effects from the cod liver oil was eliminated. After 4 weeks the egg white was increased to 40 % and a few days later to 50 %. One group of these animals received Extract A as an intraperitoneal injection. This concentrate of the potent factor[1] was given daily (6 times per week) in 0·5 ml. and later in 1 ml. portions. A small control group was given the same ration except that an extracted meat powder replaced the egg white; one of this group received injections of Eli Lilly and Company's Liver Extract No. 343.

Of these animals only the ones given the extracted meat ration without injections made any considerable gain in weight, an average of 155 g. in 9 weeks, and constituted the only group, also, in which no deaths occurred. The guinea-pig receiving the meat ration and injections of Extract No. 343 began losing from the first day of injection and died on the 20th day. The groups receiving egg white made only about one-third of the gain of the meat-fed group. There was little difference in the gains in the egg white group in respect to the presence or absence of injections of Extract A; deaths occurred in both groups about equally except that they occurred somewhat later when injections were given. Introducing linseed oil into the ration made little difference in growth and longevity.

Manifestations of the egg white disorder other than a slowing of growth were vague and slight when detectable at all and were not correlated with the presence or absence of injections. If there was a favourable effect of the potent extract, this was offset by an injurious effect of the administration of the injection; this was certainly not true in the cases of the rat, chick and rabbit. This leaves some doubt as to the specificity of the slight symptoms observed in the first experiments with guinea-pigs.

Experiments with the rabbit

Various modifications of the egg white rations which had been fed to the rat, chick and guinea-pig were tried with the rabbit with unsatisfactory results. The high mortality encountered seemed to be attributable to some deficiency or injury not related to the characteristic disorder due to egg white; there was usually extreme prostration and paralysis and often an exaggerated distention of the urinary bladder. However, in one experiment, pronounced manifestations, easily identified as characteristic of the disorder, developed in one rabbit, shown in Plate II, Fig. 1. This rabbit, however, survived only long enough for the early stages of recovery from the specific manifestations of egg white injury to occur after the administration of the potent factor. An attempt was made to improve the basal ration[2] by introducing sources of other factors which might be required by the rabbit and to eliminate a possible source of injury from the cod

[1] Extract A [Lease, 1936].

[2] This phase of the study includes experiments performed by Katherine Brusse for a thesis in partial fulfilment of the requirements for the degree of Bachelor of Science in Home Economics. Some of the data used in this paper were observations made and recorded personally by the authors on experiments of Miss Brusse, carried out under the direction of one of the authors (H. T. P.)

liver oil, in the following ration: egg white gradually raised to 40 %, bran 14·5 %, irradiated rice polishings 10 %, bone ash 1·5 %, NaCl 1 %, yeast 5 %, alfalfa 5 %, peanuts 5 %, butter fat 5 %, sucrose and starch in equal parts to make 100 %, and lettuce 15 g. daily. On this ration the animals were still growing vigorously when manifestations of the disorder became severe and while satisfactory cures were being carried out.

The order of appearance of the symptoms was somewhat different from that in the rat and chick. The first change observed was usually a slight scurf of white flakes on the skin over the shoulder blades, seen on parting the hair. The hair gradually became more dull looking and not so smooth as before, assuming a fuzzy appearance on the abdomen; soon after, the skin could be clearly seen through the thinning hair on the abdomen, along the front of the shins or on the backs of the hind paws. Then bare spots appeared on the areas mentioned as well as on the pads of the feet, and the skin became red in these spots; the long hairs dropped out in many areas not yet denuded of the fuzzy under-hair. The eyelids sometimes became glued together with a sticky exudate very early in the progress of the disorder, but more frequently at a later stage from the 25th to the 40th day. As the condition progressed, bareness extended to the back, lips, eyelids and the tail, which resembled a rat's tail, and white flaky scurf and redness of skin became more prevalent. Although the rabbit illustrated in Plate II, Fig. 1 shows some effects on the paws of a curative treatment, the pronounced symptoms which are still evident are characteristic of the rabbits on the ration just described.

In cases where the condition was alleviated by the protective factor, the first sign of recovery was the appearance, in from 1 to 2 weeks, of narrow streaks of new hair, very lustrous and dark, lying flat on the skin and seen first on the backs of the paws, the edge of the shank or the abdomen. The skin lost its redness and scurf and the eyes their soreness and exudate. Very substantial stages of improvement were secured in from 4 to 5 weeks but entire recovery required from 5 to 9 weeks. Figs. 2 and 3 of Plate II exemplify cure in a rabbit by injection of the potent factor as Extract A.

Experiments with the monkey

Two female monkeys (*Macacus rhesus*), one an adult weighing 1925 g., the other obviously quite young weighing 1180 g., were started on a ration containing 20 % of egg white, 2 % of cod liver oil, 4 % of Osborne and Mendel's salt mixture, 10 % of rice polishings, 15 % of sucrose and 49 % of cooked rice (dry weight). The egg white was raised to 30 % after several days and the sucrose and rice polishings were replaced by a 50 % ethyl alcohol extract of wheat germ and yeast. After 2 months the egg white was raised to 40 %, the rice being lowered correspondingly. Daily portions of orange pulp averaging 10 g. were given to each monkey.

For about the first 3 weeks the fur of both monkeys was sleek and glossy except that the hair of the tail was somewhat matted with faeces. Thereafter there was a progressive roughening and thinning of the hair, some bald areas appearing on the head and tail in 2 months' time. At 3 months the animals were much less active and the eyelids were distinctly red. Small white scales of epidermis appeared on the back. The monkeys frequently uttered a peculiar complaining cry although sufficient food and water was available. The activity and playfulness which they exhibited earlier were lost and they sat in a listless huddled position with the face hidden. The body weight which had increased for

the first 3 weeks of the experiment gradually tended to become constant and dropped slightly when the egg white was increased to 40 %.

In the third month of the experiment a sample of blood was drawn[1] from the femoral vein of each monkey to be used in testing for precipitins. The animals plucked at the sutures and scratched the wound vigorously and red welts appeared on the inner thigh and abdomen. These inflamed spots were at first diffuse and small but grew larger to form a continuous swollen red area. That this manifestation was a part of the physiological reaction to dietary egg white seems probable from the fact that the drawing of blood from monkeys by the same technique had been accomplished in a neighbouring laboratory several hundred times without any appearance of such an effect.[2] It may well have been analogous to the red, swollen condition of lips and paws intensified by rubbing, in rats on egg white diets.

Although these symptoms were not extreme they were considered definite enough to employ for testing their identity with egg white injury by specific curative methods. The ration was therefore changed by substituting thoroughly steamed egg white for the raw. The redness of the nose and eyelids disappeared rapidly and distinct improvement in the posture and activity of both animals gave evidence of increased well-being. The loss of hair continued, however, and although such a condition had often been observed to accompany the early stages of recovery in the rat, it was decided, after 18 days, to add 3 ml. of Extract A per monkey per day to the ration to hasten recovery. Promptly thereafter short, fluffy, thin new hair appeared on the tails of both monkeys and then on the back where the last of the old coat was being shed. In about 4 weeks the new hair was readily noticeable and gradually grew thicker, although it remained dark and fuzzy. The older monkey developed very distinct streaks of flat lustrous new hair along the thighs similar to that seen in rabbits recovering from egg white injury. The body weight which had increased slightly when the cooked egg white was substituted in the ration showed a more rapid gain during the subsequent period. The red, swollen areas on the abdomen and inner thigh, described above, receded after some weeks of therapy with Extract A but had not completely disappeared when the experiment was terminated 45 days after the initiation of the curative therapy.

There was distinct diarrhoea present in the monkeys on the egg white ration which did not disappear when the egg white was cooked or Extract A added. When the yeast-wheat germ extract was dropped from the cooked egg white ration, the diarrhoea disappeared; apparently, then, the diarrhoea was not due entirely to the egg white.

The results of the experiment would seem to establish the susceptibility of the monkey (*Macacus rhesus*) to the injurious physiological effects of dietary egg white. Although the toxic egg white ration was discontinued before sufficient opportunity had been given for the disorder to become severe, the similarity of the initial symptoms to those in the rat and rabbit and particularly the orderly disappearance of these manifestations when the egg white of the ration was steamed and a potent extract added, are convincing evidence of the essential similarity of the physiological responses in the rat, chick, rabbit and monkey.

[1] We are indebted to Mr P. H. Settlage of the Department of Psychology for taking the blood samples.

[2] Personal communication from Dr H. R. Wolfe of the Department of Zoology.

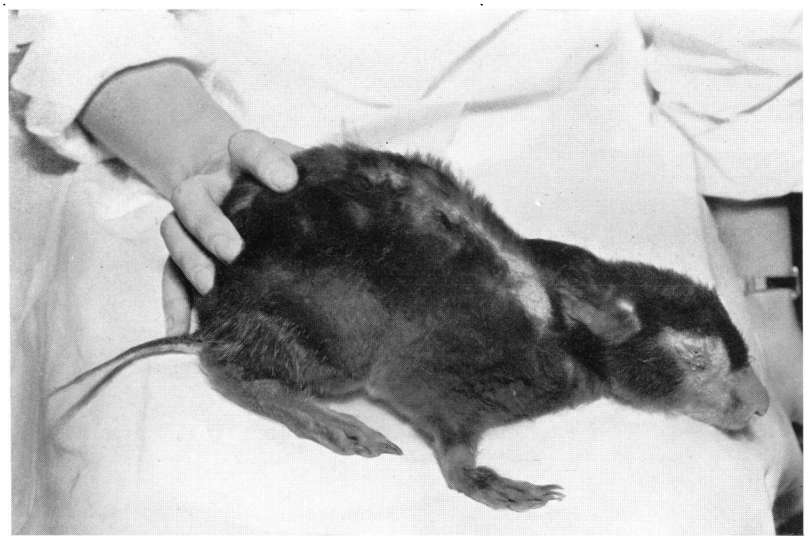

Fig. 1.

Fig. 2.

Fig. 3.

Comparison of the nutritive disorder due to egg white in the different animals

It has been shown herein that there is a definite similarity in the manifestations of the nutritive disorder due to egg white among the animals studied. Loss of hair or feathers, redness and swelling of eyelids and scaliness of the epidermis were definite or pronounced in four types and questionable in a fifth. However, in only one type, the rat, has a disorder of the nervous system been produced. While the relative degree of involvement of various parts of the body of the animals and the extent of the characteristic syndrome varied somewhat from one type of animal to another, the differences were hardly greater than those occurring among the individuals of any one type of animal as shown by such factors as acute or chronic character, swiftness of onset and the relationship of the amount of egg white to the amount of protective factor present. For example, when the onset is rapid in very young rats with little previous store of the protective factor, high concentrations of egg white cause intense swelling and redness of the lips and scaliness or soreness of the paws and even the nervous disorder to appear in advance of noticeable changes in the coat of hair; whereas mild chronic cases often show extensive denuded areas of skin before soreness or extensive dermatitis appears. We believe that this circumstance accounts for certain differences between the particular characteristics of the nutritive disorder in our laboratory and in those of others [MacKay & MacKay, 1934; Gorter, 1935; Salmon & Goodman, 1934].

Taking into consideration the extent to which the disorder is acute or chronic, the differences between the responses of four and possibly the five types of animals may very well be chiefly due to such factors as previous stores of the protective factor from varying stock rations, concentration of egg white tolerated, food intake in proportion to body weight and rapidity of growth, rather than to true species differences.

SUMMARY

1. In addition to the chick and rat, the rabbit and monkey exhibit a characteristic dermatitis when fed rations rich in egg white. The guinea-pig under similar conditions has shown only transient non-specific symptoms.

2. The symptoms are strikingly similar in the four animals exhibiting the syndrome and the differences noted may be chiefly conditioned by the more acute or more chronic nature of the disorder in the different species.

REFERENCES

Gorter (1935). *Biochem. J.* **29**, 322.
Lease (1936). *Z. Vitaminforsch.* **5**, 110.
—— & Parsons (1934). *Biochem. J.* **28**, 2109.
—— Kelly & Parsons (1936). *J. biol. Chem.* **114**, lxi.
MacKay & MacKay (1934). *J. Nutrit.* **8**, 187.
Parsons & Kelly (1933). *Amer. J. Physiol.* **104**, 150.
—— Lease & Kelly (1937). *Biochem. J.* **31**, 433.
Salmon & Goodman (1934). *J. Nutrit.* **8**, 1.

EXPLANATION OF PLATE II

Fig. 1. A rabbit exhibiting characteristic manifestations of egg white injury. Note the encrusted eyelids and the bare areas on the face, body and tail. Evidence of the curative effect of the potent extract is seen in the partial covering of the paws and tail with short hair.

Fig. 2. Hind paws and tail of a rabbit with typical pronounced egg white injury.

Fig. 3. Hind paws and tail of the rabbit in Fig. 2, 62 days after initiation of injection of Extract A. Note the thick, glossy, sleek hair which has covered the previously denuded areas.