CXCIV. HIGH PROTEIN DIETS AND ACID-BASE MECHANISM.

By MURIEL EMMA BELL¹.

*The Department of Physiology and Biochemistry, University
College, London.*

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ALTHOUGH it has been widely held, particularly since the experiments of Chittenden and of Chalmers Watson, that the presence in the food of high proportions of protein is harmful, there is still much conflict of evidence as to whether this is really so in the case of omnivorous species. Many investigators have attempted to analyse the underlying effects of increased protein rations, particularly with regard to the effect of protein in producing kidney disorders. The primary reason for undertaking the following experimental work was to determine whether the adverse effect which has been reported by some investigators was due to the acidogenic properties possessed by protein in virtue of its content of sulphur and phosphorus². This acidogenic effect has been demonstrated in man by Farquharson *et al.* [1931] who found increased excretion of ammonia and loss of calcium in response to a high protein diet. As to other effects of high protein diets in man, though such diets tend to alter the proportion of putrefactive organisms in the intestine [Sanborn, 1931; Torrer and Montu, 1931], the work of Krogh and Krogh [1913] and Thomas [1927] on eskimos, and that of McClellan and DuBois [1930], McClellan *et al.* [1930], and Tolstoi [1929] on trained subjects, all fail to indicate serious harmful effects from excessive dietary protein, whilst physical inferiority results in the case of African and of Bengali tribes if the protein is low [Orr and Gilks, 1931; McCay, 1912].

In animals the presence of excess of protein in the food has been found to cause renal hypertrophy by many workers [Osborne *et al.*, 1926–27; Hassan and Drummond, 1927; Reader and Drummond, 1925; 1926; Drummond *et al.*, 1922; Jackson, 1924–25; Jackson and Riggs, 1926; Francis *et al.*, 1931; Stewart, 1929; Longwell *et al.*, 1932; MacKay *et al.*, 1928]. Actual renal damage has been observed by Newburgh *et al.* [1919; 1923; 1928; 1931], Polvogt *et al.* [1923], Blatherwick *et al.* [1931], Maclean *et al.* [1926], Evans and Risley [1925], Smith *et al.* [1927], Jackson and Moore [1928] and Parsons [1931]. Newburgh and Curtis [1928] found that protein in the form of dried liver was particularly harmful; on the other hand Newburgh and Marsh [1925], who observed renal lesions after intravenous injection of certain amino-acids, were unable to ascribe the toxic effect to any particular compound.

According to Drummond and his colleagues and Hartwell [1928] high protein diets decrease the growth-rate of rats, whilst the reverse was claimed by Osborne and Mendel [1924]; Slonaker [1931] differentiated between the unfavourable effect of high protein intake on activity, reproduction and life-span

¹ William Gibson Research Scholar.

² In the complete oxidation of 100 g. of average protein with N : P ratio 17 and N : S ratio 14·5, phosphoric acid is produced equivalent to 30 cc. N alkali and sulphuric acid equivalent to 50 cc. N alkali.

and its favourable effect on lactation, growth of the young and freedom from tumours.

The question arises whether the discrepancies among these findings can be explained, in part at least, by the differences in the acid-base balance of the diets used by the various workers. Thus, Osborne *et al.* [1926-27] supplied vitamin B partly in the form of alfalfa which would raise the allowance of fixed base. The evil effect of dried liver might be explained by its potential acidity, whereas the protective action of unheated liver could possibly be ascribed to one of the constituents of the vitamin B complex. It was considerations such as these which led to a re-investigation of the relationship of protein, acid-base balance and vitamin B with regard to impaired growth-rate and albuminuria. It is interesting to observe that Watson [1910] found softening of the bones of his experimental rats when he fed them on meat diets without any additional alkali. These changes suggest that, in the absence of basic substances from the diet, the calcium of the bones was mobilised for purposes of neutralisation. Presumably, carnivorous animals under natural conditions cover the acid-producing elements of their meat diets by eating bones.

The rôle of common dietary salt mixtures in relation to the acid-base mechanism had been touched upon by Hassan and Drummond [1927], who found that when the growth-rate of rats was retarded by diets containing a large proportion of protein, autoclaved marmite was protective, while the ash of marmite offered no protection against the inhibitory effect on growth. A special nephrotoxic action of cystine has been assumed by Cox *et al.* [1929] and others who have observed an acute toxic nephrosis to occur when cystine is added to the food of young rats. The addition of 0.1 g. cystine means a virtual increase of 1.6 cc. of *N* acid. The question of cystine nephrosis has formed the subject of another paper [Bell, 1933, 1].

We have been in the habit of thinking that the acid-base mechanism of most animals (the rabbit, among laboratory animals, being an exception) is extraordinarily efficient, and that ammonia can readily be utilised when fixed base is deficient. However, Farquharson *et al.* [1931] report that, whenever there is a rise in the ammonia excretion there is simultaneously an increased loss of calcium.

EXPERIMENTAL.

Experiments were planned to determine whether the following diets could be improved by the addition of a mixture of sodium citrate and potassium carbonate:

1. Diets containing 70 % caseinogen.
2. Egg-white diets: (a) containing 20 % egg-white, (b) containing 66 % egg-white.

1. Diets containing 70 % caseinogen.

A. Three dozen rats were used in the first series to ascertain whether the previous experiences of this laboratory could be confirmed, *i.e.* that rats ingesting large amounts of caseinogen exhibit subnormal growth, but that the inclusion of an additional ration of yeast overcomes this retardation of growth. Three groups of rats received the following diets respectively:

	X	Y	Z
"Light white casein"	20	70	70
Dried brewer's yeast	5	5	15
Rice starch	68	28	28
Cod-liver oil	2	2	2
Salt mixture (McCollum's)	5	5	5

The average growth curves (Fig. 1) demonstrated that the diet containing 70 % caseinogen with 5 % yeast had an adverse effect on growth, but that a decided improvement occurred with 15 % yeast.

B. Several groups of rats, carefully chosen litter-mates, received diets Y and Z as above, but were further subdivided so that half of them were given enough of a potentially alkaline mixture to reduce the acidity of their urine almost to zero. The mixture of salts contained sodium citrate (10 parts) and potassium carbonate (7 parts); excessive alkalinity was avoided for fear of damaging the kidneys thereby; the urine, therefore, was kept just acid. The graphs in Fig. 2 *a* show that no improvement occurred when the alkali was added; growth of rats ingesting diet Y was still subnormal, though, probably through some seasonal variation, this set of rats grew rather more vigorously than those of Fig. 1. It appears, therefore, that 5 % McCollum's salt mixture in the diet is adequate to meet the need for fixed base when the proportion of caseinogen in the food is 70 %.

Although the majority of the animals studied in this series gave us no reason to believe that the subnormal rate of growth encountered when the diet contained 70 % caseinogen could be attributed to the acidity, one anomalous result must be mentioned in which a number of rats, obtained from a dealer, behaved rather differently. Those being fed on diet Y without alkali grew more slowly than those consuming the extra mixture of potentially alkaline salts. But as this result could not be repeated, we were compelled to regard it as purely fortuitous.

Incidentally, the graphs in Fig. 2 *b* show that the addition of alkali to a 70 % caseinogen diet containing 15 % yeast does not enhance the growth still further; nor does it produce supernormal growth when compared with the rate of growth when caseinogen constitutes 20 % of the diet.

2. Egg-white diets.

Confirmation has been obtained of the observations of Bateman [1916], Boas [1924; 1927], and Parsons [1931] that uncoagulated egg-white in the food of rats rapidly causes diarrhoea which is fatal if the diet is not amended.

In the experience of Boas with 20 % egg-white, coagulated and dried in an oven, then added to the food, rats grew normally at first, then declined and died; against this adverse effect, certain varieties of starch were protective. In the following experiments, carbohydrate was therefore supplied in the form of cane sugar, as had been done by Parsons. Chinese egg-white was prepared in one of two ways—it was coagulated and finely minced; or coagulated, dried at 80° and finely ground. Using the egg-white prepared as above, diets were compounded of:

20 % egg-white (dry basis),
68 % cane sugar,
5 % dried brewer's yeast,
5 % McCollum's salt mixture,
2 % cod-liver oil.

The severe effect reported by Boas failed to occur. There was, however, diminished growth-rate compared with that of animals ingesting food containing 20 % caseinogen (Fig. 3). No improvement was induced by adding alkali to the food. Hence, the depressed growth rate resulting from diets containing 20 % egg-white cannot be attributed to the acidogenic properties of the cystine in which it is rich.

We also repeated the experiments of Parsons, using 66 % egg-white (dry basis), with and without potential alkali, and found that no amelioration took

place when the diet included the extra base (Fig. 4). The finding that 5 % McCollum's salt mixture was adequate to cover the acid produced by the meta-

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

bolism of 66 % egg-white in the food thus dismissed the original idea of the research. What follows, therefore, is a digression in which egg-white diets are discussed.

Not only did we use egg-white, coagulated and dried at 80°, according to Parsons' method of preparation, but also egg-white coagulated and minced. Both Chinese and fresh egg-albumin gave, as Parsons found, comparable results. Care was taken not to overheat the egg-white. The food consisted of:

66 % egg-albumin (dry basis),
22 % cane sugar,
5 % salt mixture (McCollum's),
5 % dried brewer's yeast,
2 % cod-liver oil.

With our experiments, one group of rats exhibited considerable resistance to the diet; this group, charted in Fig. 4, failed to develop the acute nephritis which Parsons had observed; they grew comparatively well, and most of this group did not get the pellagra-like condition which she described. Two individual rats, however, which merely maintained their weight at about 60–80 g. for twelve weeks, were affected with the scaly and gangrenous condition of the tail, which has now been observed on a variety of diets, and which, in this laboratory, we are inclined to attribute to chronic malnutrition arising from the absence of one of the vitamin B factors.

Another set of animals, however, also receiving the 66 % egg-white diet, manifested at an early stage the symptoms of acute nephritis as described by Parsons, and most of them died (Fig. 3). The urinary findings included albuminuria, renal cells, epithelial casts, red blood cells; and in addition determinations of the blood-urea gave the high average figure of 131 mg. per 100 cc. blood. Consistently with this, the "clinical" symptoms were drowsiness and anuria, while the kidney sections showed acute nephritis. But the feature which we thought significant about the results was that one or two animals recovered without any alteration in the diet—a fact already observed by Parsons in the case of egg-white diets; similar recovery has also been reported by Cox, Smythe and Fishback in the case of cystine nephrosis. The kidney symptoms of such rats as did not die cleared up, and the animals continued to maintain their weight. The idea became insistent that the probable explanation of the train of events was that a septicaemia had occurred; that the kidneys had been incidentally affected; and that when an immunity had been established, recovery took place. An attempt was made to determine whether there was a blood infection; seven of the affected animals were killed with chloroform, and blood cultures were made, five of which were sterile, one grew a streptococcus, another grew a coliform bacillus. The results were therefore inconclusive. A possible explanation of this hypothetical septicaemia was sought in the altered conditions in the intestine, as a breeding ground for bacteria. It will be seen that the animals which were doing comparatively well on the 66 % egg-white diets exhibited irregularities in their weight curves (Fig. 4); and moreover we observed that they were subject to slight, intermittent diarrhoea. The possibility suggested itself that, for some unexplained reason, egg-white was perhaps less readily digested than other proteins, and that this might make for a greater percentage of protein degradation products in the intestine. It became interesting, therefore, to determine the percentage of nitrogen in the faeces, and it was found that excreta collected just before the rats developed nephritis contained approximately 0.06 g. N per g., whereas animals eating the stock diet were excreting 0.02 g. N per g. faeces. Later, in animals which had recovered, the quantity of nitrogen being excreted from the intestine became less—*viz.* 0.03 g., presumably as the

intestine responded by increased activity. Similar estimations of nitrogen on the excreta of rats ingesting 70 % caseinogen gave the average figure of 0.03 g.

With the presence of excessive nitrogenous material in the gut, there was the likelihood of increased numbers of putrefactive organisms in the intestine. The urine was found to give a highly positive test for indican as compared with the urine of rats being fed on the 70 % caseinogen diet. Even the rats which were doing comparatively well on the egg-white mixture always gave a strongly positive test for indican. Possibly, this gives a clue to the severity of the results of an egg-white diet as compared with other high-protein diets. The underlying effect is perhaps the presence of substances like cystine which alter the oxidation-reduction potential of the gut, and so favour the growth of specific types of organisms. Work on *in vitro* cultivation of anaerobes by Lepper and Martin [1929] has demonstrated that, for example, the addition of cooked muscle to culture medium favours the growth of anaerobes. It seemed reasonable to infer that the severe diarrhoea attendant on the administration of uncoagulated egg-albumin was only an exaggeration of this state of affairs; that since raw egg-white is not readily acted on by pepsin-HCl, as Bateman observed, there is an increased chance for bacterial multiplication in the intestine. Feldman [1920] quotes Dementjeff as stating that giving the raw whites of two eggs to a baby resulted in the appearance of indican in the urine. Recently, Nedzel and Arnold [1930-31] have shown that in the presence of uncoagulated egg-white, the permeability of the intestine to *B. prodigiosus* is increased.

Urinary examinations.

During the course of these feeding experiments, the rats were put into metabolism cages for the purpose of testing their urines for "total acidity" and for albumin. It was observed from estimations of acidity and ammonia that the rat is well able to utilise ammonia as a base. It was also obvious that considerable amounts of calcium could be absorbed into the blood stream and excreted by the kidneys; this was true whether the calcium was given as lactate or carbonate. Thus, when some stock rats were being compared with animals on the experimental diets, it was found that calcium carbonate became deposited on the funnel and urine trap, and the urine, even in the presence of toluene, became alkaline on standing. Presumably, this calcium was excreted as the bicarbonate. That rabbits can absorb calcium given as carbonate has been demonstrated by McGowan *et al.* [1931], who incorporated it in the diet and found that the rabbit's urine after standing contained a deposit of calcium carbonate. This ability of the rat and of the rabbit to absorb calcium given as carbonate from the alimentary tract seems worth mentioning in view of the belief commonly held that human beings have difficulty in absorbing calcium.

Tests for albumin in the urine revealed the fact that mature male rats normally excrete about 0.5-0.8 g. albumin per litre of urine, but that the urine of females is generally free from albumin. The results of a study on this point have been published in a separate note [Bell, 1933, 2]. Females ingesting the diets which contained a large proportion of caseinogen excreted no albumin; and no increase occurred under these conditions in the amount excreted by the males. From urinary examinations no evidence of kidney derangement was obtained.

Estimation of blood-urea.

After the animals had been fed for six months on the diet containing 70 % caseinogen, their fasting blood-urea was determined; they were fasting for six hours before being killed with chloroform. The average blood-urea for the

group receiving additional alkali was 39.9 mg. per 100 cc.; for those without alkali, the average was 41 mg.; for a control group of whose food the protein constituted 20 %, the average was 42 mg. Blood analyses by Jackson [1924-25], and by Osborne *et al.* [1926-27] showed that rats receiving high protein diets had a higher level of non-protein-nitrogen and of urea than rats ingesting normal amounts of protein; their animals were presumably not fasting. Addis, MacKay and MacKay [1926-27] have already demonstrated that the non-protein-nitrogen level quickly falls to normal when food is withheld; this extra nitrogen appears to be due, therefore, to the presence of large amounts following absorption rather than to the inability of the kidney to excrete it.

Histology.

Examination of the kidneys of rats which had received food containing 70 % caseinogen for six months failed to reveal any pathological changes. According to Newburgh, it requires a longer period than six months' feeding with excessive caseinogen to produce degenerative changes.

SUMMARY.

1. The experience of those workers is confirmed who find that rats exhibit a subnormal rate of growth when fed on diets consisting largely of caseinogen and containing the usual proportion of yeast serving as the source of the vitamin B complex, and who also find that the inclusion of further proportions of yeast supplement renders the high-caseinogen diet adequate for growth.

2. The addition of potential alkali to diets containing a large percentage of caseinogen or coagulated egg-white does not improve the growth-rate. The presence of 5 % McCollum's salt mixture in the diet is therefore sufficient to meet the need for fixed base when high protein diets are given.

3. No evidence of kidney damage has been obtained in rats which have been fed for six months on diets containing 70 % caseinogen.

4. The presence of uncoagulated egg-white in the diet of rats is rapidly fatal, as has been found by Bateman and others.

5. Subnormal growth occurred as a result of feeding rats with diets containing 20 % coagulated egg-white, but the decline in weight reported by Boas failed to occur.

6. Subnormal growth also resulted when the food included 66 % egg-white; some of the animals developed acute nephritis and died. The findings of Parsons are therefore confirmed.

7. Examination of the faeces for nitrogen and of the urine for indican in the case of animals receiving egg-white diets led to the belief that excessive putrefaction in the intestine occurs when these diets are given.

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REFERENCES.

- Addis, MacKay and MacKay (1926-27). *J. Biol. Chem.* **71**, 152.
Bateman (1916). *J. Biol. Chem.* **26**, 263.
Bell (1933, 1). *Biochem. J.* **27**, 1267.
— (1933, 2). *J. Physiol.* **79**, 191.
Blatherwick, Medlar, Connolly and Bradshaw (1931). *J. Biol. Chem.* **92**, lxxxiv.
Boas (1924). *Biochem. J.* **18**, 422, 1321.
— (1927). *Biochem. J.* **21**, 712.
Cox, Smythe and Fishback (1929). *J. Biol. Chem.* **82**, 95.
Drummond, Crowden and Hill (1922). *J. Physiol.* **56**, 413.
Evans and Risley (1925). *J. Amer. Med. Assoc.* **84**, 1870.
Farquharson, Salter, Tibbetts and Aub (1931). *J. Clin. Invest.* **10**, 221.
Feldman (1920). Ante-natal and post-natal child physiology, p. 475. (Longmans, Green and Co., London.)
Francis, Smith and Moise (1931). *Amer. J. Physiol.* **97**, 210.
Hartwell (1928). *Biochem. J.* **22**, 1212.
Hassan and Drummond (1927). *Biochem. J.* **21**, 652.
Jackson (1924-25). *Proc. Soc. Exp. Biol. Med.* **22**, 482.
— and Moore (1928). *J. Clin. Invest.* **5**, 415.
— and Riggs (1926). *J. Biol. Chem.* **67**, 101.
Krogh and Krogh (1913). A study of the diet and metabolism of the Eskimos. (Bianco Luno Copenhagen.)
Lepper and Martin (1929). *Brit. J. Exp. Path.* **10**, 327.
Longwell, Hill and Lewis (1932). *J. Nutrition*, **5**, 539.
McCay (1912). The protein element in nutrition. (Edward Arnold, London.)
McClellan and DuBois (1930). *J. Biol. Chem.* **87**, 651.
— Rupp and Toscani (1930). *J. Biol. Chem.* **87**, 669.
McGowan, Cunningham and Auchinachie (1931). *Biochem. J.* **25**, 1295.
MacKay, MacKay and Addis (1928). *Amer. J. Physiol.* **86**, 459.
Maclean, Smith and Urquhart (1926). *Brit. J. Exp. Path.* **7**, 360.
Nedzel and Arnold (1930-31). *Proc. Soc. Exp. Biol. Med.* **28**, 358.
Newburgh (1919). *Arch. Int. Med.* **24**, 359.
— (1923). *Arch. Int. Med.* **32**, 850.
— and Curtis (1928). *Arch. Int. Med.* **42**, 801.
— and Johnston (1931). *J. Clin. Invest.* **10**, 153.
— and Marsh (1925). *Arch. Int. Med.* **36**, 682.
Orr and Gilks (1931). The physique and health of two African tribes. *Med. Res. Coun. Rep. Ser.* No. 155, 92.
Osborne and Mendel (1924). *J. Biol. Chem.* **59**, 13.
— — Park and Winternitz (1926-27). *J. Biol. Chem.* **71**, 317.
Parsons (1931). *J. Biol. Chem.* **90**, 351.
Polvogt, McCollum and Simmonds (1923). *Bull. Johns Hopkins Hosp.* **34**, 168.
Reader and Drummond (1925). *J. Physiol.* **59**, 471.
— — (1926). *Biochem. J.* **20**, 1256.
Sanborn (1931). *J. Infect. Dis.* **58**, 37.
Slonaker (1931). *Amer. J. Physiol.* **98**, 266.
Smith, Moise and Jones (1927). *Proc. Soc. Exp. Biol. Med.* **24**, 746.
Stewart (1929). *Vet. J.* **85**, 209.
Thomas (1927). *J. Amer. Med. Assoc.* **88**, 159.
Tolstoi (1929). *J. Biol. Chem.* **83**, 747.
Torrer and Montu (1931). *J. Infect. Dis.* **49**, 144.
Watson (1910). Food and feeding in health and disease. (Oliver and Boyd, London.)