CLXXI. CYSTINE AND NEPHROTOXICITY.

By MURIEL EMMA BELL¹.

*From the Department of Physiology and Biochemistry,
University College, London.*

(Received July 6th, 1933.)

By administering excessive cystine to rats and other animals many workers appear to have produced injurious effects on the kidneys. This damage has not always been observed however, and it seemed possible that considerable clinical importance might attach to a study of the reason of the variability of the results.

The earlier experiments in which cystine was given to animals were concerned primarily with a study of the fate of ingested cystine. Wohlgemuth [1903-04] appears to have observed no toxic effect in rabbits after oral administration of 5 g. suspended in water. Blum [1904] reports a number of experiments in which cystine was given to dogs in large amounts, and in most of his cases nephrotoxicity was not observed. Abderhalden and Samuely [1905] gave as much as 8 g. of dileucylcystine subcutaneously without producing renal damage. Later, however, Abderhalden [1922] noted that kidney injury accompanied the deposition of crystals of cystine in the renal tubules of the mouse after ingestion of free cystine. Similarly, Lignac [1926] demonstrated that cystine injected in the form of a suspension into mice reappeared in crystalline form in the convoluted tubules. Lewis [1925] introduced 0.5-1.0 g. cystine per kg. in alkaline solution by stomach tube into rabbits every second or third day. After a few doses, there were definite signs of renal injury. Newburgh and Marsh [1925, 1, 2] injected solutions of amino-acids into rabbits and dogs and found that cystine was one of those which were nephropathic. Curtis and Newburgh [1926; 1927] added cystine to the diet of the rat, observing that large doses caused severe nephritis and death in a few days; smaller amounts caused moderate injury in the course of a few months, whilst 1.5 % produced necrosis of the tubules in one year. Cox, Smythe and Fishback [1929] reported acute toxic nephrosis in young rats under 60 g. in weight ingesting 0.3-0.9 % free cystine; some of the animals, however, recovered from their acute illness and proceeded to grow normally without any alteration in the ration. Cox and Hudson [1929-30] extended these observations and obtained evidence that cystine nephrosis might be prevented by increasing the allowance of vitamin B concentrate. The rats exhibited variable resistance to the nephrotoxicity of cystine, and the susceptibility was attributed to hereditary factors rather than to the influence of the stock diet. Addis, MacKay and MacKay [1926], on the other hand, had obtained no evidence of renal impairment from blood-urea determinations, urinary or histological examinations in rats which had lived one-third of their life-time on a diet containing 1 % cystine. The proportion of dried yeast included in their

¹ William Gibson Research Scholar.

diet was 10 %. Recently, Longwell, Hill and Lewis [1932] studied the effect of 0.3–0.6 % of cystine in the diet of young rats receiving either no yeast at all or a 2 g. equivalent of yeast extract. They report “complete absence of evidence that a diet high in cystine produces pathological changes in the kidneys.” This was true even of some rats which were in poor condition at the beginning of the experiment. Renal hypertrophy due to absence of yeast was no greater in the presence of cystine.

The insolubility of cystine is a factor which complicated those of the above-mentioned experiments in which cystine was injected parenterally. There are obvious objections to the method of injecting a suspension of cystine or a quantity of an alkaline solution of cystine.

In the matter of the feeding experiments, the positive findings probably cover two states of affairs; there is the acute condition from which, as Cox and co-workers report, the animals recover without change in the ration and the more slowly produced lesions of the type reported by Newburgh.

One question which naturally presents itself is whether the effect of cystine is due to its acidogenic properties. The intention in the experiments herein reported was to determine whether the effects of cystine could be overcome by regulating the proportion of acid to base in the diet. That the quantity of acid produced by the oxidation of cystine is appreciable can be seen by calculating that the addition of 0.1 g. of cystine means a virtual increase of 1.6 cc. of *N* acid. The effect of this increased acidity would be more marked in the case of rabbits than in rats, for the former animals have little power of calling on ammonia to neutralise acids. Arising from the acidogenic qualities of cystine was the question of available base; it was necessary to find out whether the salt mixtures ordinarily employed in compounding synthetic diets were adequate in base content to cover this increased production of acid. The results reported in this contribution were obtained in conjunction with a study of the acid-base mechanism in relation to excessive protein feeding, reported elsewhere [Bell, 1933].

EXPERIMENTAL.

It was decided to attempt to produce the condition of cystine nephrosis in very young rats, such as had been reported by Cox and co-workers, and see whether the addition of potential alkali to the diet prevented its incidence. The diet was compounded of:

				%
“Light white casein”	...	...	...	20
Rice starch	...	...	...	68
Dried brewer's yeast	...	...	...	5
Salt mixture (McCollum's)	...	...	...	5
Cod-liver oil	...	...	...	2

As the quantity of cystine, which in the experience of Cox, Smythe and Fishback was sufficient to induce nephrosis, ranged from 0.3 to 0.9 % of the diet, we began by using 1.0 %, but as no derangement occurred, it was decided very early to raise it to 2.0 %. Even when the rats were only 35 g. in weight when cystine feeding commenced, there was no evidence of cystine nephrosis. The rats were kept in metabolism cages, and the urine was examined daily. One group received enough of a mixture of potassium carbonate (7 parts) and sodium citrate (10 parts) to reduce the acidity of the urine almost to neutrality. The addition of potential alkali did not augment the growth rate—it was already

maximum (Fig. 1). Since no effect from the cystine feeding was obtained, the experiment was terminated at the end of 4 weeks. The fasting blood-urea was determined and was found to be normal in both groups; the average for the animals receiving cystine was 38 mg. per 100 cc.; that for the animals being given alkali as well as cystine was 43 mg. Sections of the kidneys failed to give any evidence whatever of inflammatory changes.

Fig. 1. Average growth curves of rats ingesting 1 and 2 % cystine.

↑ = 1 % cystine in diet;
 ↑↑ = 2 % cystine in diet;
 C + A = Cystine + alkalisising salt.

It is difficult to understand why cystine *per se* should be harmful to the kidneys, unless it is due to insolubility or to acid production. Still more difficult is it to understand why, in spite of its insoluble and acidogenic nature, the animals should recover without change in the ration. If cystine itself exerts a nephropathic action, we should expect to find a high incidence of nephritis in cystinuria, but in the following references in the literature to uncomplicated cases of cystinuria, no mention is made of findings that indicate that the excretory tissue is involved; cystinurics have usually been discovered either accidentally or because of symptoms of renal calculus [Garrod, 1923; Smillie, 1915; Looney, Berglund and Graves, 1923; Lewis and Lough, 1929; Robson, 1929; Brand, Harris and Biloon, 1930]. Certainly, the last-named authors have found that there is excreted, in this disorder of metabolism, a cystine complex which decomposes in the urine with liberation of free cystine; but even so, we should expect that if this liberation of the amino-acid begins early enough to form a stone in the pelvis of the kidney, the process has already begun soon after the formation of the glomerular filtrate.

The variety of kidney affection induced in young rats by excessive cystine feeding seems to be some indirect effect, irregular in its occurrence, and temporary in its influence. The recovery without alteration in the diet seems to suggest that the nephritis is part of an infection. It is possible that the presence of the cystine in the intestine brings about an alteration in the oxidation-reduction potential of the contents of the gut and so favours the growth of anaerobes. Quastel and Stephenson [1926] have demonstrated that the addition of cystine to culture media favours the growth of anaerobes by altering the oxidation-reduction potential. The condition of "pulpy kidney" in lambs seems to have some features in common with cystine nephrosis, and "pulpy kidney" has

recently been ascribed by Bennett [1932] to infection by the *B. ovitoxicus*, an organism allied to the Welch bacillus. If cystine nephrosis is part of an infection in which the obvious and fatal effects are due to kidney involvement, the recovery can be explained by the development of an immunity.

SUMMARY.

Feeding young rats with diets containing 1.0–2.0 % free cystine has failed to produce the condition of “cystine nephrosis.” It is suggested that the condition reported by other investigators to occur in young animals is probably due to an infection, to which an impetus is given by the altered oxidation-reduction potential in the intestines.

Grateful appreciation is herewith expressed to the Royal Society of Medicine for the scholarship which has enabled me to make these observations, to the Medical Research Council for defraying the cost of materials and to Prof. J. C. Drummond for helpful advice and criticism.

REFERENCES.

- Abderhalden (1922). *Pflüger's Arch.* **195**, 204.
— and Samuely (1905). *Z. physiol. Chem.* **46**, 187.
Addis, MacKay and MacKay (1926). *J. Biol. Chem.* **71**, 139.
Bennett (1932). *Bulletin 57, Council for Sci. and Ind. Res. Commonwealth of Australia*.
Bell (1933). In the press.
Brand, Harris and Biloon (1930). *J. Biol. Chem.* **86**, 315.
Blum (1904). *Hofmeister's Beiträge*, **5**, 1.
Cox, Smythe and Fishback (1929). *J. Biol. Chem.* **82**, 95.
— and Hudson (1929–30). *J. Nutrit.* **2**, 271.
Curtis and Newburgh (1926). *J. Clin. Invest.* **2**, 611.
— — (1927). *Arch. Int. Med.* **39**, 817.
Garrod (1923). *Inborn errors of metabolism.* (Oxford Univ. Press.)
Lewis (1925). *J. Biol. Chem.* **65**, 187.
— and Lough (1929). *J. Biol. Chem.* **81**, 285.
Lignac (1926). *Krankheitsforsch.* **2**, 43.
Longwell, Hill and Lewis (1932). *J. Nutrit.* **5**, 539.
Looney, Berglund and Graves (1923). *J. Biol. Chem.* **57**, 515.
Newburgh and Marsh (1925, 1). *Arch. Int. Med.* **36**, 682.
— — (1925, 2). *J. Amer. Med. Assoc.* 1703.
Quastel and Stephenson (1926). *Biochem. J.* **20**, 1125.
Robson (1929). *Biochem. J.* **23**, 139.
Smillie (1915). *Arch. Int. Med.* **16**, 503.
Wohlgemuth (1903–04). *Z. physiol. Chem.* **40**, 81.