

*Brief Communication*

THE INTERDEPENDENCE BETWEEN HIGH CADMIUM  
CONTENT OF HORSE KIDNEY CORTEX AND THE FALSE  
POSITIVE KIDNEY TEST FOR THE DETECTION OF  
ANTIBIOTIC RESIDUES AT MEAT INSPECTION

It is generally known by veterinary meat inspectors that when horse kidney cortex samples are tested for the presence of inhibitory substances they quite often give a positive reaction without known preslaughter use of antibiotics or chemotherapeutic substances (*Tiainen* 1968).

It has also been known since 1958 (*Vallee*) that the horse kidney cortex has an exceptionally high cadmium content. The aim of the present study is to show the interdependence between these two peculiarities.

*Materials and methods*

Fresh kidneys were obtained from 19 horses from the local slaughter-house. All the horses were apparently healthy prior to slaughter. After samples had been taken from the kidney cortexes for cadmium analysis, the kidneys were kept frozen until analysed for the presence of inhibitory substances.

Sixteen male Sprague-Dawley rats weighing about 400 g each were given 2 mg cadmium per kg body weight s.c. as cadmium chloride in 2 ml sterile water.

Six similar rats, the controls, were given 2 ml sterile water s.c. After seven days the rats were sacrificed by decapitation. The anterior halves of the left kidneys were analysed for cadmium content. The posterior halves were kept frozen until analysed for the presence of inhibitory substances.

The cadmium analyses were done by flameless atomic absorption with an optimum working range between 0.01 and 0.1 mg cadmium. The apparatus used was a Perkin-Elmer 303 atomic absorption spectrophotometer equipped with a graphite furnace and graphite cell power supply HGA 72.

The testing of the horse kidney cortexes for the presence of inhibitory substances was done by the method of Lorenzen (1967). The test microbe was *Sarcina lutea* ATCC 9341. To test the inhibitory properties of cadmium, cadmium chloride solutions were made in distilled water with a cadmium concentration of 20, 30, 40, 50, 60, 70, 80 and 100 mg Cd/l; 0.1 ml each of these solutions was pipetted onto separate filter-paper disks ( $\varnothing$  12.7 mm) and placed on the agar surface inoculated with the test microbe together with the cylindrical horse kidney cortex samples. The rat kidney halves were minced before testing for the presence of inhibitory substances. The test was performed as described for milk (Juncher *et al.* 1950), using the same test microbe as for the kidney cortex samples. In this method cylindrical wells in the test substrate are filled with the material to be tested. In addition to the minced kidneys one well was filled with distilled water and two with cadmium chloride solutions corresponding to 20 and 100 mg Cd/l.

### Results

The cadmium content of the 19 horse kidney cortex samples varied between 30.7 and 108.0 mg/kg wet weight, mean  $59.0 \pm 21.9$  mg/kg (m.  $\pm$  s). The mean cadmium content of the kidney halves obtained from rats given the cadmium chloride injection was found to be  $57.67 \pm 15.03$  mg/kg wet weight (range 40.7—112.1). The kidney halves of the control rats contained from 0.006 to 0.229 mg Cd/kg wet weight, with a mean of  $0.117 \pm 0.078$  mg/kg. All of the horse kidney cortex samples inhibited the growth of the test microbe. The diameter of the inhibitory zone was roughly proportional to the measured cadmium concentrations, especially when the zone diameters corresponding to the lowest and highest cadmium concentrations were compared. The lowest concentration of cadmium in the cadmium chloride-impregnated filter paper disks that inhibited the growth of the test organism was 30 mg/l. The zone diameter was found to be proportional to the cadmium concentration. Inhibition of growth of the test organism was shown around the wells filled with the kidney material obtained from the rats given the cadmium chloride injection and around the wells filled with cadmium chloride solutions, but not around the wells filled with kidney material from the control rats or with distilled water.

### Discussion

The cadmium in horse kidneys is mainly bound to a small-size protein, metallothionein, mol. wt. about 10000 (Kägi & Vallee 1961). The same holds true for rat kidneys a few days after a cadmium injection (Wisniewska et al. 1970). Because of the relatively small size of this protein it obviously readily diffuses into an agar medium and thus inhibits the growth of the test organism. All the kidneys tested originated from relatively old horses; it was not possible to obtain kidneys from younger horses which, because of the long biological half-life of cadmium (Friberg et al. 1971), might be expected to have a lower cadmium content in their kidneys. Nevertheless the present study indicates that the positive reactions obtained from horse kidney cortices when tested for the presence of inhibitory substances at meat inspection may be due to the cadmium content.

T. J. Pekkanen

The Department of Food Hygiene, College of Veterinary Medicine,  
Eija-Riitta Seuna and Ruth Stabel-Taucher  
The State Veterinary Medical Institute, Helsinki, Finland.

### REFERENCES

- Friberg, L., M. Piscator & G. F. Nordberg: Cadmium in the environment. C. R. C. Press, the Chemical Rubber Co. Cleveland, Ohio 1971, 166 pp.
- Juncher, H., I. Magnusson & O. Rømer: Målinger af penicillinindholdet i mælk efter intramammære infusioner af penicillinholdige præparater. (The determination of penicillin content of milk after intramammary treatment with penicillin preparations). Nord. Vet.-Med. 1950, 2, 765—788.
- Kägi, J. H. R. & B. L. Vallee: Metallothionein: A cadmium and zinc containing protein from equine renal cortex. II. Physicochemical properties. J. biol. Chem. 1961, 236, 2435—2442.
- Lorenzen, P.: Anwendung und Auswertung des Antibiotikatestes in der bakteriologischen Fleischuntersuchung. (The use and the evaluation of the test for the detection of antibiotic residues at meat inspection). Arch. Lebensmitt.-Hyg. 1967, 18 (2), 30—32.
- Tiainen, O. A.: Bakterikasvua estäviä aineita hevosen munuais-kudoksessa. (Inhibitors to bacterial growth in horse kidney). Suom. Eläinlääk.-L. 1968, 74, 308—309.
- Vallee, B. L.: Metalloenzymes: Correlation of composition, structure and function. Proc. 4th Int. Congr. Biochem., Vienna 1958, 138—166.
- Wisniewska, J. M., B. Trojanowska, J. Piotrowski & M. Jakubowski: Binding of mercury in the rat kidney by metallothionein. Toxicol. appl. Pharmacol. 1970, 16, 754—763.

(Received June 6, 1974).

Reprints may be requested from: Timo J. Pekkanen, College of Veterinary Medicine, 55006 Helsinki, Finland.