much greater than in sheep fed clover; shapes varied from irregular plates about 2μm diameter to needle-like structures 50μm long; a sheet of silicised plant cells 60μm long and 40μm wide was also seen. Occasional OPs up to 50μm long were seen in digests of kidneys.

There is evidence that particles of dietary origin, of considerably greater size than those seen in the present study, can pass from the alimentary tract to body fluids and tissues. Volkheimer and Schulz (1968) and Volkheimer et al (1969b) studied the persorption of various particles through the intestinal wall and transport via blood and lymph into tissues, including the kidney. They found that the maximum sizes of quartz sand particles, starch granules and metallic iron transported in this way were 150μm, 110μm and 52μm respectively. Pontefract and Cunningham (1973) injected asbestos fibres into rats' stomachs and subsequently found particles in tissues. These were generally 0.2 to 2.0μm long but some were as large as 15μm. One fibre, 24μm long, was seen in the blood.

Graminaceous plants are generally accepted as containing 10 to 20 times more silica than legumes and other dicotyledons. Not infrequently they contain in excess of 2% silica, most of which in mature or dried plant material, for example hay and chaff, is in the solid form as OPs and these solid particles pass through the intestinal tract undigested (Jones and Handreck 1967). Consequently larger numbers are available for persorption from cereal than from clover diets and this may be the reason for the difference in numbers of OPs in tissue on the 2 diets in this investigation. That this is not the only reason is suggested by the fact that whereas considerable difficulty was experienced sectioning tissues from the sheep fed chaff and grain (required for the primary investigation with these sheep) due to hard particles which chipped the microtome knives, this is not our common experience with animals that have received intervention so conditioned the alimentary tract as to facilitate persorption. The effects of previous lush clover diet, a period of starvation, and stress of transport could have been as important in this respect as those found for drugs and diet by Volkheimer and Schulz (1968) and Volkheimer et al (1969a), who suggested that these factors increased the motility of the intestinal wall, which resulted in increased persorption.

The suggestion (Baker et al, 1961) that OPs may be a factor in the formation of urinary calculi is supported by this demonstration of their occurrence in kidney tissue and it would be of interest to determine the factors affecting the persorption of OPs, and the relationship between their presence in kidney tissue and the occurrence of urinary calculi.

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References

ISOLATION OF MAREK'S DISEASE HERPESVIRUS OF LOW PATHOGENICITY FROM COMMERCIAL CHICKENS

Previous studies indicate that apathogenic or low-pathogenic strains of Marek's disease herpesvirus (MDHV) exist in commercial chickens (Rispens et al 1972; Cho and Kenzy 1972). Virus strains of varying pathogenicity — acute, classical and apathogenic types — may exist in single flocks or in small groups of chickens within a flock (Biggs and Milne 1972). Little is known about the prevalence of apathogenic field strains of MDHV in Australia. This paper reports the isolation of MD virus of low pathogenicity from commercial chickens.

Three 6-month old White Leghorn pullets which were serologically positive for Marek's disease (MD), as determined by the agar-gel precipitation (AGP) test (Chubb and Churchill 1968), were obtained from a commercial flock in Sydney. The birds and their parents had not been vaccinated with turkey herpesvirus (HVT) and clinical MD was unknown in the flock. They were killed at different times within a month after their arrival at this laboratory and their serums were retested for MDHV precipitating antibody with positive results. At autopsy no gross lesion of MD was noticed in internal organs of any of these chickens. Separate primary cell cultures of kidney (CK) from the 3 chickens were prepared by the conventional procedures of trypsinisation and using tricine-buffered medium 199 supplemented with inactivated calf serum at the concentrations of 5% for growth and 2% for maintenance medium.

Herpes-type cytopathic effect (CPE) was noticed in all 3 cultures between the sixth and the eighth day post-seeding. It consisted of small compact plaques comprising small round refractile cells. On further incubation, the plaques increased in size and the affected cells in the centre of the plaques detached forming a central hole. On passage in embryo fibroblasts of chick (CEF) and duck (DEF), the plaques were larger and less compact than those in CK cultures. Generally the isolates grew slowly, particularly in DEF cultures, and using tricine-buffered medium 199 supplemented with 5% calf serum at the time of infecting CEF monolayers prevented development of plaques, indicating that the cytopathetic agents were sensitive to an inhibitor of DNA synthesis. One of the isolates (strain 7120) was plaque-purified through 3 cycles of propagation in DEF monolayers under agarose (0.5%) overlay, sonicated, Millipore-filtered and lyophilised (Calnek et al 1970). The lyophilised preparation retained infectivity, as determined by subsequent subculturing in CEF and DEF cultures. The foci developing in DEF monolayers overlayed with...
agreed were greyish white and measured 0.3 to 0.5 mm in diameter.

Infected cell lysates stained negatively with phosphotungstic acid showed particles with the morphology of herpesvirus. Not infrequently the CPE failed to develop in DEF cultures but virions were consistently seen by electron microscopy. On subsequent passage, however, characteristic CPE developed and herpesvirus particles were seen in cell lysates.

The pathogenicity of the plaque-purified strain was tested by inoculating subcutaneously 1,000 focus-forming units of cell-associated virus into 20 day-old chickens free of maternal antibodies to HVT and MDHV. Ten uninoculated birds of the same age served as controls. These chickens were derived from hens of an experimental flock raised in the laboratory and previously known to be MD susceptible as judged by the development of MD lesions in progeny inoculated with the blood from chickens with clinical MD. Ten inoculated and control chickens were seen for up to 8 weeks of age. No gross lesion characteristic of MD was noticed in any of these chickens. Histologically a few focal aggregates of lymphoid cells were seen in the perirenal, liver, sciatic nerve or in kidneys of some inoculated chickens. The CK cultures from the infected birds showed CPE characteristic of MDHV. By the AGP test, employing a known MD-positive antiserum, the antigen prepared from the infected CK cultures showed lines of identity with a known MD antigen. The control birds proved negative by these tests. However, in agreement with the findings of Eidson and Anderson (1971), HVT antibody did not react with the MD antigen in the AGP test. The remaining 10 inoculated chickens were observed for up to 16 weeks when they were killed. None showed clinical signs or gross lesions of MD but their sera contained MDHV precipitins.

The foregoing characteristics of the isolates indicated that they were MDHV with low pathogenicity similar to those previously described (Biggs and Milne 1972; Cho and Kenzy 1972). These and other studies (Jackson personal communication) indicate that MDHV strains that are apathogenic or of low pathogenicity are widespread in the poultry population in Australia. Such field strains need to be investigated for use as an alternative MD vaccine for breeding flock in order to obviate the frequent "pour on" treatments. The status of field strains of MDHV that are apathogenic or of low pathogenicity in the evolutionary scale of avian herpesviruses is unclear. The possibilities are that they originate independently or they emerge by selection and/or adaptation from the pathogenic MDHV in hosts with genetic and age resistance, or under immune stresses. The finding of avirulent MDHV attenuated by laboratory manipulations (Churchill et al. 1969) seems to negate the first hypothesis. Similarly the status of HVT remains undetermined but the microscopic lymphoproliferative lesions, similar to those of MD, in nerves and gonads of HVT-inoculated chickens (Witter et al. 1976) suggest the oncogenic potential of this virus. It seems, as suggested (Witter et al. 1970; Kaleta and Bankowski 1972), that the 3 types of MDHV—acute, classical and apathogenic, and HVT have a common phylogenetic origin. While they vary in pathogenicity, they retain antigenic kinship. The final answer to this question awaits future study.

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COW FERTILITY AFTER LICE TREATMENT WITH FAMPHUR

The widespread use of "pour on" insecticides to control cattle lice infestation has led to occasional reports of infertility. While investigation of individual cases often reveals more likely explanations for the low fertility, occasional convincing reports have been received from farmers. Since treatment for lice control would often be carried out near mating time in autumn calving herds, we conducted an experiment to investigate the effects of frequent "pour on" insecticide application during mating.

A mixed herd of 53 cows with calves at foot was divided according to breed type and age of cow into 2 similar groups.

The treated group consisted of 27 cows. Over the mating period these cows received 5 applications of 60 ml of 13.2% W/W Famphurst at fortnightly intervals. The first treatment was applied 1 week before the start of mating.

The control group was comprised of 26 cows which did not receive insecticide treatment.

The 2 groups were grazed together. After each application of Famphurst the groups were held in separate yards for at least 3 hours before being returned to pasture.

* Warbex, Cooper Australia Ltd., Melbourne.