TABLE II

Infectious virus, CF antigen and interferon titre in brains of West Nile virus infected suckling mice

WN virus strains	Dose inoculated Dex LD_{50}/ 0·03 ml	Virus titre Dex LD_{50}/ 0·03 ml	Titre of M.Br. CF antigen	Interferon* titre IU/ml
68856	2·6	7·5	1 : 256	13887
G 2266	3·0	6·2	1 : 128	11678
G 2266	2·7	6·6	1 : 128	10339
672698	2·8	6·5	⩾1 : 256	6730
P 4230	2·7	7·0	1 : 128	6694

* Geometric mean of three separate assays.

sensitivity of different strains to interferon induced during multiplication[11]. Only one correlation that could be derived in the study was between interferon inducing ability and size of plaques on Vero cell line[8]. Human strains (672698, P 4230) which produced large size plaques (1·5 to 2·9 mm in diameter) were poor inducers of interferon. Whereas bat and mosquito strains which produce small size plaques (0·3 to 0·9 mm) were found to be efficient inducers of interferon This observation lends support to the suggestion by Umrigar and Pavri[8] that bat strain (68856), might be a good inducer of interferon in mice. The correlation between interferon inducing ability and plaque size in Vero cell line, a heterologous system, is difficult to assess. Moreover, Vero cell line has been shown to be incapable of production of interferon[12]. Period of isolation and passage level also did not seem to influence variation observed in interferon titres. Cole and Wisseman[13] have observed that low, medium and high mouse brain passaged lines of dengue type I human isolate did not differ significantly in rate of virus multiplication or amount of interferon induced in suckling mice brains.

Finter[14] has reported that brains of adult mice inoculated with an unnamed strain of WN virus are a rich source of mouse brain interferon (Geometric mean titre 9,500 units/ml). Earlier, Vainio *et al.*[10] and Haahr[15] have studied interferon induction by E 101 strain of WN virus. All the studies mentioned above have employed adult mouse brain as source of interferon and have not included international reference preparation of interferon in their assay. Hence, it is difficult to compare the present results with those reported earlier.

The present study has revealed that out of five strains, three isolated from non-human sources induced higher amount of interferon as compared to human isolates. Further, there seems to be no clear relationship between biological properties of five strains of WN virus and quantitative ability to induce interferon in suckling mouse brains. The observation that human strains of WN virus are less efficient in iinduction of interferon in brains of suckling mice needs further study.

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CHELATING AGENT EDTA DECREASES THE TOXICITY OF COPPER TO FISH

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COPPER toxicity is well investigated in the field of toxicology because the pollutant has adverse effect on water quality, fish production and aquatic ecosystem[1–4]. Furthermore, even trace concentrations of copper (one tenth to the twentieth of the accepted standards for drinking water) can be lethal for fish in soft water[5].

It is practically impossible to prevent the waste discharge of this metal from several industries into water at low concentrations. However, the disodium salt of ethylene-diamine tetracetic acid (EDTA) can be used for reducing the fish mortality at short-term exposure.

Static bioassays with copper alone and copper plus EDTA, were carried out in the laboratory. Test fish were common guppy (*Lebistes reticulatus* Peters) of 0·15 g and 1·5 cm total length. There were three batches of ten fish in each bioassay and procedure for physico-chemical analysis of the test water were the same as reported[6]. The water characteristics of the test solutions were as follows : pH 7·3; temperature 16·5° C; alkalinity 160 ppm as $CaCO_3$; total hardness 230 ppm as $CaCO_3$; dissolved oxygen 7 ppm and conductivity 950 μ mhos per cm. Tests were terminated after 96 hr, and individual times for the death of the fish were recorded. The LC 50's (median lethal concentration at which 50% mortality appeared) and their 95% confidence limits were calculated by moving-average-angle method[7]. Copper was added as reagent grade $CuSO_4 . 5H_2O$ but concentrations are given as ppm of Cu^{++}. Concentrations of the chelating agent are also given in ppm of disodium salt of EDTA.

The fish mortality at different periods of exposure in static bioassays of copper solution, and copper plus EDTA are shown in Table I. Metal concentration of 5·1 ppm and above caused 100% fish mortality in 96 hr. At 1·28 and 0·64 ppm of Cu alone in the 96 hr of exposure, 77 and 20% mortalities were observed. EDTA caused a marked decrease in copper toxicity. At 2·56 ppm of Cu (or below) no fish mortality was observed with EDTA addition. Survival rate of fish was also increased, for example at 7·68 ppm of Cu alone, the median period of survival (LT 50) was 8 hr while with the addition of 5 ppm EDTA, the LT 50 (median lethal time) value was 48 hr and with the 10 ppm of EDTA it was 22 hr. Toxicity of all copper concentrations decreased with EDTA addition but much lower overall toxicity was observed in presence of 5 ppm of EDTA. No fish died in controlled container with or without EDTA.

The LC 50's and their 95% confidence limits with and without EDTA are given in Table II. At 24, 48 and 96 hr, the confidence limits do not overlap in copper alone and copper plus EDTA bioassays. Therefore, the LC 50's at these periods of exposure significantly differ. The experiment exhibited a progressive decline of LC 50's from 24 hr to 96 hr indicating that the toxicity of copper increases with time. The 96 hr LC 50's suggested that with the addition of 5 and 10 ppm, of EDTA the toxicity decreased by a factor of 6·46 and 3·49 respectively.

Present study clearly demonstrates a reduction of copper toxicity due to the complex formed by EDTA. These data also agree with the other chelating agents which protect salmonid fish against copper–zinc toxicity[8]. Several "anti-pollutants" such as sodium citrate, sodium thiosulphate, etc., were used for the reduction of toxicity of copper salts[2], but EDTA was

TABLE II

The LC 50 values and their 95% confidence limits for copper in ppm

Time (hr)	Copper alone	5 ppm EDTA salt added	10 ppm EDTA salt added
24	3·96 (3·21–4·61)	>20·48	8·66 (7·98–9·28)
48	3·38 (2·40–3·86)	10·19*	4·70 (4·47–5·23)
96	1·23 (0·95–1·65)	7·95 (7·28–8·29)	4·30 (4·04–4·35)

* 95% confidence limits could not be calculated.

TABLE I

Fish mortality at different periods of exposure with copper alone and copper plus EDTA. No mortality was observed in 5 and 10 ppm of EDTA alone

Conc. Cu (in ppm)	Copper alone		5 ppm EDTA added to each conc. of Cu				10 ppm EDTA added to each conc. of Cu		
	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr
20·48	100	100	100	40	90	100	100	100	100
10·24	100	100	100	10	50	80	40	70	100
7·68	100	100	100	10	20	50	60	100	100
5·12	73	83	100	..	..	..	10	40	80
2·56	10	47	90	..	..	..	..	..	..

the best on account of its stronger chelating properties[8]. Nishikawa and Tabata[9] have observed that the reduction in toxicity is related to the stability constants of the metal complex formed.

The results of the present study suggest that EDTA can be used as an anti-pollutant for temporary treatment of copper pollution. EDTA has the ability to wrap itself around Cu ion and coordinate with all six octahedral position at once. The chelating properties of EDTA are so remarkable that it can even remove the Cu atom from an enzyme[10].

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OCCURRENCE OF *BEAUVERIA ALBA* ON A SPIDER

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THE spider belonging to the genus *Achaearanea* occurring in the city of Madras was found to harbour a fungus. The fungus was found inside the abdomen of young (immediately after hatching) and adult spiders of both sexes as white patches (Fig. 1). The fungus was isolated and identified as *Beauveria alba* (Limber) Saccas.

The spider was washed twice in sterile distilled water, surface sterilized in 0·1% mercuric chloride, washed again in sterile water and placed in an agar slant and

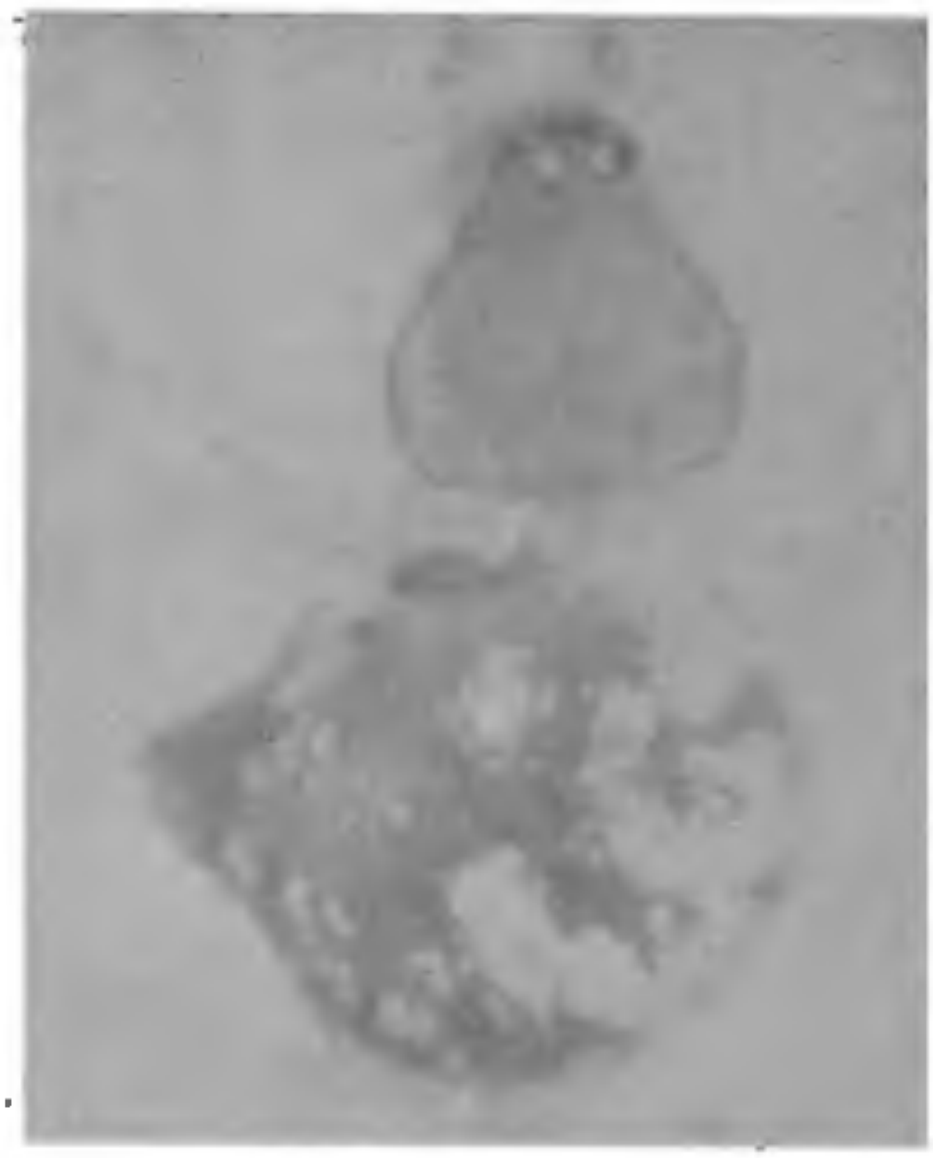

FIG. 1. *Achaearanea* showing fungal patches in the abdomen, × 30.

its abdomen crushed with a sterile needle. Potato dextrose agar, potato sucrose agar or Czapek's agar slants were tried. Slants thus prepared were incubated at 30 ± 1°C. The fungus appeared as white growth on the 6th day of incubation on PDA and on the 10th day on the other two media. There was no sporulation on any of the media even after 15 days of incubation. On exposure to near-ultra violet light from 2 BLB lamps (Sylvania Black Light Lamps 40 W) the fungus sporulated on PDA. The fungal colony was lanose to floccose and hyaline. The medium was not coloured. Conidia were hyaline, smooth, globose to subglobose.

Species of *Beauveria* are insect parasites[1,2], but *B. alba* has been isolated from the cover of a book, skin lesion and sputum of human and from *Pisum* sp[3]. Our report appears to be the first one of this fungus occurring on a spider. The specimen has been deposited at CMI, Kew, Surrey, England (IMI 250309).

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