this to be a desirable level for compounds of unknown activity.

Toxicity: Three of the drugs were fed to uninfected mice at doubling concentrations in the diet, and the method of REED and MUENCH[11] was used to calculate the projected concentration resulting in 50% mortality over a 7-day period (LD_{50} – 7 days). The values obtained were: rafoxanide 0.038%; oxyclozanide > 0.4%; nitroxynil 0.163%. The toxicity of diamphenethide was not tested but could be expected to be very low[12].

Chemotherapeutic effect: Five periods of medication covering the whole of the immature phase of migration of the parasite were used. Activity was based on comparison of parasite recovery (fluke burden) of medicated versus infected unmedicated control (IUC) groups for a particular period, as determined by piece-meal dissection of the liver. Percent reduction of flukes equals the IUC value minus the medicated group value divided by the IUC value $\times 100$, where the values are in terms of number of flukes per animal. During the medication period, IUC animals consumed a mean of 4.5 g of feed per mouse per day (range 4.0 to 5.1), while medicated feed was consumed at a mean of 4.6 g per mouse per day (range 4.1 to 5.7).

Results. The results will be given for each drug separately below and are shown in the Table.

Rafoxanide: It was active for all doses used for all periods except –1 to 6 days post infection. Complete elimination of all parasites was seen for the periods 7 to 21, 14 to 21, and 21 to 31 days post infection. It was inactive when given from days –1 to 6 post infection and highly active only at 0.025% for the period 7 to 14 days post infection.

Oxyclozanide: It was active at the dosages tested for the periods 7 to 21, 14 to 21, and 21 to 31 days post infection but only at the higher dose level for the 7- to 14-day period. It was inactive when given at 0.1% for –1 to 6 days post infection.

Nitroxynil: It was active for all periods tested except –1 to 6 days post infection. It was less active for the period 7 to 14 days post infection than for later periods.

Diamphenethide: It was active for all periods tested. This was the only drug that exhibited a marked degree of activity against the very early stages of *F. hepatica* when given in the feed at 0.1% for the period –1 to 6 days post infection. There was a marked fluke reduction for the period 7 to 14 days post infection, with a lesser degree of reduction for the 14- to 21-day post infection period.

Discussion. It is evident from the results that the fasciolicidal activity of the drugs tested was clearly shown in a *F. hepatica* mouse system. Though not shown by the data, it is also notable that flukes recovered from medicated animals were generally retarded in size, and there was an obvious reduction in gross liver pathology that paralleled drug activity. As the drugs tested are the 4 most prominent, new fasciolicidal agents available, it is considered that the mouse-*F. hepatica*-system should lend itself well to the detection of such activity in unknown compounds. Thus, murine fascioliasis could be used for the screening of potential fasciolicidal agents, and the drugs used in this study could be used as standards in such testing. In fact, a method for the primary empirical screening of chemicals for activity against *F. hepatica*, utilizing mice medicated from 14 to 21 days post infection and changes in pathologic status as a parameter of activity, has recently been presented[13].

Zusammenfassung. Die Wirkung von vier fasziloziden Agentien (Rafoxanid, Oxyklozanid, Nitroxynil und Diamphenethid) wurde im Mäuseversuch verglichen. Alle vier Substanzen waren gegen unreife *Fasciola hepatica* wirksam und entfalteten ihre höchste Wirksamkeit gegen spätere unreife Stadien, mit Ausnahme von Diamphenethid, welches gegen die Frühstadien der unreifen Parasiten am stärksten war.

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Molecular Weight of Virus-Induced Tortoise Interferon in Cell Cultures

In an earlier work[1] we reported the interferon production in cell cultures of tortoise (*Testudo graeca*) kidney after infection with arboviruses (Semliki Forest, West Nile). In addition to the physico-chemical characteristics of the tortoise interferon, an investigation was carried out to determine its molecular weight. For this purpose, the method of Sephadex gel filtration was used.

The 'crude' interferon was obtained by infecting primary monolayer cell cultures of tortoise kidney (TGK) with Semliki Forest virus (SFV), and subsequent treatment of the cultural liquid harvested at the 48th h after the virus inoculation, by technics previously described[1]. After a 72 h dialysis against 30 volumes of PBS, pH 7.4, the interferon preparation was concentrated 15–30 times with polyethyleneglycol 6000 (Carbowax 20M, Serva).

The molecular weight was determined by column chromatography in Sephadex gel according to the method of ANDREWS[2,3]. A column 110×1.5 cm was packed with hydrated Sephadex G-100 beads (Pharmacia, Uppsala) in PBS, pH 7.4, containing 0.02 sodium azide, and left for 48 h at 10 °C to achieve equilibration. Calibration of the column was performed with the following proteins used as standard markers, with known molecular weight: human serum albumin (69,000) Koch-Light, ovalbumin (45,000), soybean trypsin inhibitor (21,500) Miles-Seravac, cytochrome c (13,000) Miles-Seravac. Proteins were dissolved in PBS and layered in 2 ml volume, containing a mixture of human serum albumin and soybean trypsin inhibitor 3 mg each and ovalbumin and cytochrome c 2 mg each. 0.2% Blue dextran 2000 (2,000,000) Pharmacia, Uppsala, was used as an additional marker to determine the exclusion volume of the column. The flow rate was about 7 ml/h/cm^2. 4-ml-effluent fractions were collected with fraction collector Nowator 301 B (Poland).

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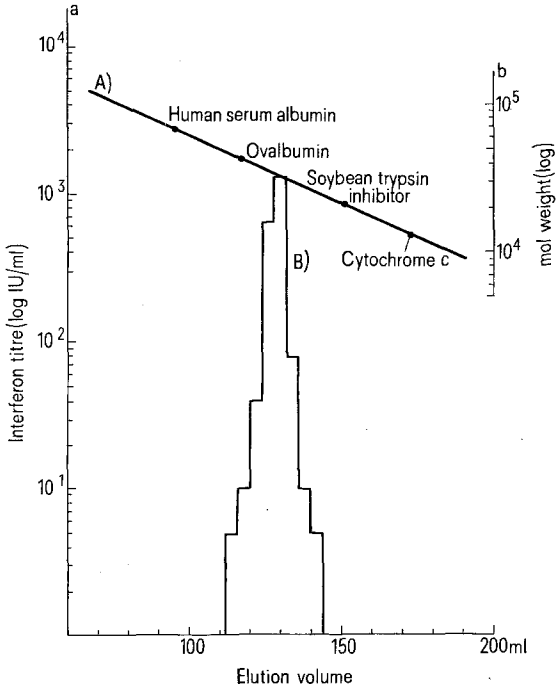
Optical density of the fractions was read spectrophotometrically (spectrophotometer SFD-2, USSR) at a wavelength of 280 nm; blue dextran was additionally read at 625 nm, and cytochrome c at 412 nm.

2-ml sample of the concentrated interferon preparation, containing blue dextran and cytochrome c was layered on top of the column. Interferon titre in interferon units (IU) per ml of the 4-ml effluent fractions was determined according to CPE inhibition method in tube monolayer TGK cell cultures, using vesicular stomatitis virus, strain Indiana, as challenge virus[1].



Chromatography of SFV-induced tortoise interferon on Sephadex G-100 column. A) Calibration curve with use of reference proteins (standard markers). B) Elution profile of 1 ml concentrated interferon preparation.

The results of the chromatography on Sephadex G-100 column of the interferon preparation, obtained from SFV infected TGK cells, are presented in the Figure. As can be seen, a single peak of interferon activity was established. A molecular weight of 33,500 $\pm$ 4.5 % of the virus-induced tortoise interferon was calculated by plotting the elution volume of the interferon peak on the calibration curve of the column.

Evidently, the determined molecular weight of the interferon of tortoise, class Reptilia, is within the 25,000–38,000 range found by other authors for virus-induced interferons in cell cultures of birds[4–6] and mammals[7–9].

By using isoelectric focusing in polyacrylamide gels for purification of mouse and human interferons, CARTER[10] obtained a dissociation of the native interferon molecule which he assumes to be a dimer of similar or identical subunits (with molecular weght of 19,000 or 12,000, respectively). Our study on the tortoise interferon by the same technique is in progress.

Zusammenfassung.. Das Molekulargewicht des Interferons, durch Selmiki Forest Virus in Zellkulturen von Schildkrötenniere induziert, wurde mittel Gelchromatographie mit Sephadex G-100 bestimmt und es ergab sich ein Wert von 33,500.

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Quantitative Ultrastructural Differences in the Mitochondrium of Pleomorphic Bloodforms of *Trypanosoma brucei*[1,2]

In a previous study[3] we investigated the quantitative alterations occuring in the organelle content of a pleomorphic strain of *Trypanosoma brucei* (STIB 33) during the transformation of the slender to the stumpy form. In this context, distinct differences in the volume density of the mitochondrium (V_{Vmi}) were shown to exist between slender and stumpy forms. The increased V_{Vmi} for the stumpy forms was found to correlate with morphological and biochemical observations on the mitochondrial changes in bloodstream trypanosomes[4,5].

The present paper describes our investigations of the changes in the surface densities of the inner (S_{Vmii}) and outer (S_{Vmio}) mitochondrial membranes.

Material and methods. The strain of *T. brucei* (STIB 33) and the preparation methods for electron microscopy have already been described[3]. The same 7 series with determined frequency of slender forms (f(SF), Table I) were used for the present study. In each series, 4 blocks were randomly chosen and thin sections cut in 1 section plane. From 1 section per block 40 micrographs were taken by systematic random sampling on 35 mm rollfilm at a primary magnification of 7,300 × in a Philips EM 300. The resulting 160 micrographs per series were contact-printed and the positive films projected on the screen of a table projector[6] at a final magnification of about 83,000 ×. The screen contained a square lattice test system, the distance between the points measuring 2 cm (equivalent to 0.241 μm on the section).

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