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Effect of Parenteral Liver Extract on the Fission Rate of *Paramecium caudatum.*

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The antipernicious anemia or hemopoietic liver principle possesses the well-known property of stimulating the production and maturation of red blood cells, leucocytes, and blood platelets. Consequently, we studied the effect of parenteral liver extract on the rate of fission of another type of cell; *viz., Paramecium caudatum.* If the fission rate of this organism were significantly increased by the liver principle, the possibility of utilizing the procedure as a bioassay method for the antipernicious anemia principle suggested itself.

The method of determining the fission rate of *Paramecium caudatum* was essentially that of Jennings[1] as modified by Middleton,[2] and involved the daily isolation of the organisms and the determination of their fission rates by direct observation at 24-hour intervals. The antipernicious anemia liver extract was used in the form of a parenteral liver extract labelled 3 cc derived from 100 g of liver and now labelled 10 units per 3 cc.* The culture medium used was a 0.05% solution of malted milk in distilled water. The fission rates of *Paramecium caudatum* were determined for periods ranging from 5 to 50 days in concentrations of liver extract in the malted milk solution, varying from 0.1 to 0.00005% on the basis of fresh liver equivalent. The minimum effective concentration in pernicious anemia patients is about 0.01% of liver equivalent. Suitable controls with liver extract inactivated by heating at 100°C for 24 hours and with the plain malted milk solution were employed. For each determination, 20 specimens of paramecium were employed.

The results outlined in Table I show that in all of the concentrations used, liver extract failed to produce a consistent significant increase in the fission rate of *Paramecium caudatum.* In the 6 highest concentrations of liver extract and in all of the concentrations of inactivated liver extract, there was a significant decrease in the fission rate. Statistical analysis of the results obtained during 5 successive

1 Jennings, H. S., *J. Exp. Zool.*, 1913, **14**, 279.

2 Middleton, A. R., *J. Exp. Zool.*, 1915, **19**, 451.

* Supplied by Dr. Guy W. Clarke, Lederle Laboratories, Inc., Pearl River, New York.

TABLE I.
Effect of Active and Inactivated Parenteral Liver Extract on the Fission Rate of *Paramecium caudatum*.*

Conc. in % of liver equivalent	No. of days obser-vation	Avg No. of fissions per line for the 20 lines of each experiment: Active liver extract	Heated liver extract	Malted milk controls	Excess† of controls
.1	5	2.1 ± .104		3.6 ± .074	1.5 ± .127
.05	5	1.8 ± .081		3.8 ± .108	2.0 ± .108
.025	5	3.2 ± .010		3.6 ± .111	0.4 ± .012
	16	11.3 ± .199		14.2 ± .098	2.9 ± .222
.013	16	12.0 ± .168		16.0 ± .162	4.0 ± .234
.006	16	13.0 ± .126		15.0 ± .137	2.0 ± .186
	10	9.0 ± .075		9.2 ± .060	0.2 ± .096
	16	14.6 ± .113		15.7 ± .113	1.1 ± .159
.003	50	35.3 ± .262		38.1 ± .340	2.8 ± .429
	65		34.3 ± .3986	48.6 ± .279	14.3 ± .487
	10	9.2 ± .060		8.3 ± .069	—0.9 ± .606
.0016	50	37.1 ± .318		38.1 ± .340	1.0 ± .466
	65		35.8 ± .552	48.6 ± .279	12.8 ± .736
	10	9.4 ± .719		8.3 ± .069	—1.1 ± .715
.0008	50	34.7 ± .373		31.7 ± .432	—3.0 ± .515
	65		35.9 ± .455	48.6 ± .279	12.7 ± .497
.0004	50	33.8 ± 1.272		31.7 ± .432	—2.1 ± .728
	65		35.8 ± .545	48.6 ± .279	12.8 ± .612
.00005	50	33.8 ± .561		31.7 ± .432	—2.1 ± .708
	65		37.3 ± .612	48.6 ± .279	11.3 ± .454

* 3 cc derived from 100 g of liver.
† Differences which are statistically significant are underscored.

10-day periods of the 50- and 65-day observations with active and inactive liver extracts failed to reveal any progressive stimulation or retardation of the fission rate.

Conclusions. In the range of its effective concentration in the body

of the pernicious anemia patient, parenteral liver extract does not significantly change the fission rate of *Paramecium caudatum*. In the higher concentrations employed, liver extract decreased the fission rate. Inactivated liver extract decreased the fission rate in all concentrations used. Obviously the procedure has no value as a bioassay method for the antipernicious anemia principle.

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Amino Acid Nitrogen in Urine of Children with the Nephrotic Syndrome Following Intravenous Amino Acids.

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Recently Farr and MacFadyen[1] reported observations on the concentration of amino acids in the blood plasma of children with the nephrotic syndrome. They observed that while the disease persisted the concentrations were subnormal. Furthermore, nephrotic crises[1, 2] were ushered in with a sudden further fall in plasma amino acid concentration and clinical recovery from these crises was attended by a rapid rise to the precritical subnormal concentration. The close association of the clinical course of the disease with changes in the amino acid content of the blood plasma suggested the possibility of treating these patients, particularly during nephrotic crises, by giving amino acids intravenously. A prerequisite to this therapy was the determination of urinary loss of amino acid by *nephrotic* children following intravenous amino acid injections. The present study was designed to bear on this point.

Four children with the nephrotic syndrome on the wards of the hospital were chosen for study. Two were males, W. O'B. and J. C., 5 and 6 years of age respectively, and 2 were females, E. S. and R. Q., both 7 years of age. These children had all been studied extensively during the period of their hospitalization which varied from 7 to 14 months. At the time of the study all had characteristically low plasma proteins, normal hemoglobin, normal blood urea nitrogen, normal or elevated urea clearances, normal blood pressure and absence of hematuria. All were free from obvious acute or focal infection.

1 Farr, L. E., and MacFadyen, D. A., *Am. J. Dis. Child.*, in press.
2 Farr, L. E., *Am. J. Dis. Child.*, in press.