

spectra were made, as well as analysis of the acetyl derivative of the compound.

This isolated  $\beta$ -sitosterol was estrogenically active when gradual doses were injected in groups of 10 immature female mice (NMRI-IIan). The uterine response to increasing levels of  $\beta$ -sitosterol indicated no relation between its dose and the estrogenic activity (Table). Based upon the mouse-uterine-weight test, 2.0  $\mu$ g represented the minimum daily dose per mouse when injected s.c. Under the same conditions, a dose response curve was constructed with 17- $\beta$ -estradiol (Schering) from which the estrogenic potency of the partially purified extract and the isolated active principle was estimated. As expressed in terms of estradiol equivalents, the extract and  $\beta$ -sitosterol were  $9.68 \times 10^{-5}$  and  $7.71 \times 10^{-2}$  respectively.

The relatively high potency of  $\beta$ -sitosterol is very marked compared with the other identified phytoestrogens. BICKOFF et al.<sup>9</sup> mentioned that coumestrol is 30

times as active as genistein which is  $4.53 \times 10^{-5}$  estradiol equivalents<sup>10</sup>. According to WONG and FLUX<sup>11</sup>, the relative potencies of genistein, biochanin A and diadzin are 1.5:1.0:0.4, a result which differed from that of CHENG et al.<sup>12</sup> giving the greatest activity in diadzin, an equal activity in biochanin A and genistein, and least in formononetin. In this connection, a study of the literature emphasizes a great difference in opinion regarding estrogenic potency of the individual phytoestrogens. But this is not astonishing if we consider the variables, e.g. location, temperature, plant variety, experimental animals, estrogenic test, etc., which affect estrogenicity in plants<sup>13, 14</sup>.

**Zusammenfassung.** 21 Tage alten weiblichen Mäusen wurde während dreier Tage durch s.c. Injektionen ein Extrakt von Sabalfrüchten (*Serenoa repens* Small, früher *Sabal serrulatum* Schult) verabreicht. Es wurde eine hohe Östrogenaktivität festgestellt, welche durch  $\beta$ -Sitosterol hervorgerufen wird, welches in dieser Pflanze in relativ hoher Konzentration vorhanden ist.

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Estrogenic activity of shrub palmetto fruits and its isolated active principle

Group No.	Daily dose ( $\mu$ g)	Average body wt. (g)	Uterine wt. as % of body wt. (mg/100 ml)	P value
Control				
1	oil	9.2 $\pm$ 0.275	69.5 $\pm$ 2.94	—
Crude extract				
2	10,000	10.2 $\pm$ 0.304	65.5 $\pm$ 2.05	$\approx$ 0.20*
Partially purified extract				
3	2500	10.6 $\pm$ 0.331	83.9 $\pm$ 1.86	$\approx$ 0.0027
4	5000	10.5 $\pm$ 0.396	92.4 $\pm$ 1.81	< 0.0002
$\beta$ -sitosterol				
5	1.0	11.2 $\pm$ 0.320	73.0 $\pm$ 3.75	$\approx$ 0.735*
6	2.0	12.2 $\pm$ 0.256	85.1 $\pm$ 4.12	$\approx$ 0.01
7	5.0	11.2 $\pm$ 0.357	80.8 $\pm$ 2.29	$\approx$ 0.01
8	10.0	11.5 $\pm$ 0.392	83.2 $\pm$ 3.85	$\approx$ 0.02
9	25.0	12.5 $\pm$ 0.354	82.0 $\pm$ 1.47	< 0.005
10	50.0	9.9 $\pm$ 0.314	81.2 $\pm$ 2.58	$\approx$ 0.01

The mean value ( $\pm$  S.E.) of 10 animals in each group. \* Insignificant difference between experimental and control mice.

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<sup>11</sup> E. WONG and D. S. FLUX, *J. Endocrin.* 24, 341 (1962).

<sup>12</sup> E. CHENG, L. YODER, C. D. STORY and W. BURROUGHS, *Science* 120, 575 (1954).

<sup>13</sup> E. M. BICKOFF, *Oestrogenic Constituents of Forage Plants* (Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire, USA 1968).

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## Transmission of Rauscher Leukemia in Mice

Rauscher Leukemia Virus (RLV) produces a rapid erythroid leukemia and develops lymphoid leukemia in mice surviving the abnormal erythrocytopoiesis<sup>1-4</sup>. The experiments presented in this communication are concerned with the transmission of RLV in mice.

The stock of RLV was obtained from spleen extracts from leukemic mice supplied by Dr. F. J. RAUSCHER of the National Cancer Institute, Bethesda, Maryland, and from plasma from leukemic H<sub>a</sub>/ICR swiss mice in our laboratory. The experimental procedures employed in these experiments are similar to those previously described in our studies with Friend virus<sup>5-7</sup>.

Table I presents data showing our attempt to transmit RAUSCHER leukemia (RL) to newborn H<sub>a</sub>/ICR swiss by various body fluids, tissue extracts, and feces from RAUSCHER virus-infected female and male H<sub>a</sub>/ICR swiss

mice. All specimens were collected from mice when viremia was at its peak, during the first month after infection.

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<sup>2</sup> R. F. ZEIGEL and F. J. RAUSCHER, *J. natn. Cancer Inst.* 32, 1277 (1964).

<sup>3</sup> R. ZIEGLER and M. A. RICH, *Cancer Res.* 24, 1406 (1964).

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<sup>6</sup> E. A. MIRAND, J. T. GRACE JR. and R. F. BUFFETT, *Nature* 209, 696 (1966).

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Body fluids that produce RL in newborn mice when inoculated with RLV were blood (95-98% incidence), milk (84%), urine and semen (5%). Saliva gave negative results, tissue extracts of spleens gave an incidence of 88-92%, liver extracts 89-96%, and brain 6%-7%.

Table I. Transmission of RAUSCHER leukemia (RL) to new-born Ha/ICR swiss mice by various body fluids and by tissue and feces extracts

Types of specimens inoculated into new-born mice	Specimens from infected females <sup>a</sup>		Specimens from infected males <sup>a</sup>	
	No. RL/No. inoculated	% pos.	No. RL/No. inoculated	% pos.
(A) Body fluids				
Blood	38/40	(95%)	41/42	(98%)
Milk	21/25	(84%)	—	—
Saliva	0/18	(0%)	0/37	(0%)
Semen	—	—	2/40	(5%)
Urine	0/42	(0%)	3/56	(5%)
(B) Tissue extracts				
Spleen	36/41	(88%)	55/60	(92%)
Liver	23/24	(96%)	48/54	(89%)
Brain	2/36	(6%)	5/75	(7%)
Embryo	0/84	(0%)	—	—
(C) Feces extract	0/84	(0%)	0/51	(0%)

<sup>a</sup> Infected females and males inoculated i.p. with 0.2 ml of 10<sup>-1</sup> splenic filtrate containing RLV. Specimens were obtained from these mice when viremia was at its peak and 0.05 ml of 10<sup>-1</sup> filtrate from these specimens was injected s.c. into new-born mice.

Embryo extracts were negative. It appears that transplacental transmission of RLV is unlikely or that it is not the most effective route of transmission. Extracts of feces from infected mice gave negative results.

Table II. RAUSCHER leukemia (RL) in offspring reciprocally foster-nursed on Ha/ICR swiss

Group	No. offspring in litter with RL	Age of leukemic mice at death (months)
I Offspring from normal swiss females foster-nursed on infected RL swiss mothers	5/12 3/10 1/12 0/12 4/12 0/12	3, 5½, 6, 6, 7½ 5, 7½, 8 4½ — 4, 6½, 7, 7½ —
II Offspring from infected RL swiss females foster-nursed on normal swiss mothers	0/11 0/12 0/9 0/12 0/8 0/12 0/12	

Animals in Groups I and II were observed for 18 months. Spontaneous mammary tumors were evident in some survivors after one year. This is characteristic for this strain. Spontaneous leukemia was below 0.5% during this period.

Since a high incidence of the disease was obtained with milk, reciprocal foster-nursing experiments were carried out to determine if this was the most effective route of vertical transmission. Table II indicates that this is the case, for 13 of 69 offspring from normal Ha/ICR swiss mice foster-nursed on infected swiss mothers had RAUSCHER leukemia. The earliest development of RL was seen at 3 months. Offspring from infected mothers foster-nursed on normal females failed to develop the disease. Littermates injected with RLV were kept in continuous contact with non-injected littermates. None of the non-injected littermates developed RL (Table III).

Table III. Contact transmission of RAUSCHER virus disease in Ha/ICR swiss

Number in litter	Number injected with RLV <sup>a</sup>	Number not injected with RLV
10	5 (5)	5 (0)
12	6 (6)	6 (0)
9	5 (5)	4 (0)
8	4 (4)	4 (0)
11	6 (5)	5 (0)
12	6 (4)	6 (0)
12	6 (6)	6 (0)
10	5 (5)	5 (0)
11	6 (6)	5 (0)
10	5 (5)	5 (0)

<sup>a</sup> New-born mice received 0.05 ml s.c. of 10<sup>-1</sup> splenic filtrate containing RLV and were kept in the same cages as non-injected littermates. ( ) = Number infected with RL.

From these studies one can conclude that RL has a pattern of transmission similar to that of Friend virus disease<sup>5-9</sup>. The most effective route of transmission is vertical via the mother's milk. This was determined by injecting milk from RL females and by reciprocal foster-nursing experiments. Transplacental transmission of RL was not achieved in these studies. Moreover, horizontal transmission of RL in natural environment, even under optimal conditions, appears to be minimal<sup>10</sup>.

*Zusammenfassung.* Es wird gezeigt, dass die wirkungsvollste Übertragung des RAUSCHER-Leukämie-Virus vertikal durch die Milch geht. Eine transplacentale Übertragung wurde nicht erreicht. Die horizontale Übertragung hingegen war unter optimalen Bedingungen minimal.

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