giant cells and extensive cellular destruction in CE cells but did not visibly affect HA cells. Multinucleated giant and spindle-like cells were observed in cultures of HA cells infected with a low passage human amnion adapted strain, which did not visibly affect CE cells. With agar overlay technic viral plaques were obtained with both strains in susceptible cell monolayers.

1. Milovanovic, M. V., Enders, J. F., Mitus, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 120.
2. Katz, S. L., Milovanovic, M. V., Enders, J. F., *ibid.*, 1958, v97, 23.
3. Enders, J. F., *Diagnostic Procedures for Virus and Rickettsial Diseases,* Am. Public Health Assn., New York, 1956, 281.
4. Vogel, J., Shelokov, A., *Science,* 1957, v126, 358.
5. Weller, T. H., Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 124.
6. Watson, B. K., *ibid.,* 1952, v79, 222.
7. Watson, B. K., Cheever, F. S., *J. Immunol.,* 1955, v75, 161.
8. Wolff, H. L., *Nature,* 1955, v176, 604.
9. Taylor, C. E., *J. Immunol.,* 1953, v71, 125.
10. Brandt, C. D., *Virology,* 1958, v5, 177.
11. Enders, J. F., *Ann. Rev. Microbiol.,* 1954, v8, 473.
12. Takemoto, K. K., Lerner, A. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 179.
13. Henle, G., Deinhardt, F., *ibid.,* 1955, v89, 556.
14. Deinhardt, F., Henle, G., *J. Immunol.,* 1957, v79, 60.
15. Brandt, C. D., *Virology,* 1961, v14, 1.

Received June 22, 1961. P.S.E.B.M., 1961, v107.

Toxic Effects of Potato Sprouts and of Solanine Fed to Pregnant Rats. (26762)

B. E. KLINE, H. VON ELBE, NORMAN A. DAHLE AND S. MORRIS KUPCHAN
(Introduced by H. P. Rusch)
Wisconsin Alumni Research Foundation and Department of Pharmaceutical Chemistry, University of Wisconsin, Madison

During one phase of a long-time feeding study involving reproduction in rats(1), some of the animals were fed a potato diet containing an estimated 7% of sprouts (wet wt.). On this diet, several of the rats delivered dead pups or pups that died within 2 or 3 days. The adults showed no toxic symptoms except, possibly, a lactation deficiency, and successfully raised subsequent litters after the sprouts had been eliminated from the diet.

The present report describes studies undertaken to confirm the toxic effects of sprouts and to isolate and identify the toxic factor involved. Exploratory tests confirmed the toxicity of the sprouts, both in a synthetic basal diet and in ground lab chow, when they were fed at a 10% level. When a sample of sprouts was dried at 50°, ground, and extracted successively with petroleum ether and methanol, the toxic factor was found to have been concentrated in the methanol extract. The methanol extract was found to be rich in alkaloids (Mayer's test). Furthermore during preliminary attempts at fractionation of the components of the methanol extract followed by biological tests, it was noted that those fractions which were exposed to mineral acid treatment lost an appreciable proportion of their toxicity. The latter observations led to the hypothesis that a glycosidic alkaloid such as solanine might be the toxic factor involved.

The general toxicity of sprouted potatoes and of solanine is well documented(2-5). However the literature appears to contain no studies of the effects of sprouted potatoes or of solanine on reproduction of animals. Consequently, potato sprouts in the frozen state were acquired for further sprout feeding tests and for isolation of solanine for inclusion in the biological tests. Preliminary tests indicated that the aglycone solanidine was inactive, and the latter alkaloid was therefore not included in the studies reported below.

Isolation of solanine. Frozen potato

sprouts (6.12 kg) were allowed to melt overnight at room temperature. The weight of the melt was 2.247 kg. The weight of damp sprouts was 3.930 kg. The sprouts were dried in a forced-air oven at 65° for 24 hours and ground; weight of ground sprouts, 802 g. Extraction of the dried sprouts was effected essentially according to the procedure described by Prelog and Jeger(6). The dried sprouts (400 g) were macerated with occasional stirring with 2% acetic acid (2 l) for 30 hours. The marc was separated by suction filtration and washed with 2% acetic acid (four 500 ml portions). The filtrate and washings were combined and alkalinized to pH 11 with concentrated ammonium hydroxide. The basic solution was allowed to stand for 12 hours at room temperature followed by 2 hours in the ice chest, and the crude alkaloid was separated by centrifugation. After washing twice with 2% ammonia solution (60 ml), the crude alkaloid was dried in a desiccator overnight (weight, 6.3 g). Exhaustive extraction with ether in a Soxhlet extractor dissolved 1.08 g. The ether-insoluble solid was crystallized from 80% ethanol, yielding 1.18 g (0.038%) of crystalline solanine, m.p. 278° after sintering from 269°. Recrystallization from 80% ethanol yielded 738 mg (0.024%) of solanine m.p. 279° after sintering from 270°: $[\alpha]_D^{30}$ −58° (c 4.00 pyr.). Reported m.p. 285°, $[\alpha]_D$ −56.5, −60° (pyr.)(6). Extraction of the aqueous melt afforded only a trace of additional alkaloid.

Methods. Holtzman rats approximately 4 months of age were mated, one male to 4 females. As soon as pregnancy was indicated by increase in weight, the females were placed in individual cages and fed one of the following diets.

I	Basal	(ground commercial lab chow)
II	"	+ 10% ground frozen sprouts (wet wt.)
III	"	+ 30 mg/kg solanine*
IV	"	+ 40 mg/kg solanine*
V	"	+ 30 mg/kg solanine†

* Solanine obtained from Mann Research Laboratories, Inc., N. Y., and from L. Light and Co., Ltd., Colnbrook, Bucks, England.

† Solanine isolated from the frozen sprouts.

TABLE I. Effect of Potato Sprouts and Solanine on Survival of Rat Pups.

Group	No. litters	Pups		% pups weaned	No. zero litters*
		Born	Weaned		
I	11	115	95	82.6	1
II	9	83	42	50.6	5
III	10	100	31	31.0	6
IV	10	106	33	31.1	5
V	4	41	8	19.5	2

* Litters in which all pups died.

Some of the rats were on the test diets for only a few days before dropping their first litter. They were then kept on the test diet until they had a second litter. The rats ate the diet readily; no food consumption records were kept.

Results. The toxic effect of the test diets is indicated in the Table which gives, for each diet, number of litters, total number of pups born and weaned, percent of total pups weaned, and number of litters in which all pups died. Most of the deaths occurred within 3 days of birth and were evidently caused by starvation.

Group II contained 2 rats which raised one litter, and then a second litter while eating the sprout diet. None of the pups in the other 5 litters of this group survived to weaning age.

Group III contained one rat which successfully raised 2 litters on the test diet.

Discussion. The results of this feeding test indicate that the toxicity of potato sprouts to pregnant rats is due to the alkaloid solanine. The exact nature of the toxicity has not been determined. However, it was observed that the pups that died did not have milk in their stomachs. One attempt was made to foster nurse control pups on a solanine-fed mother. This was unsuccessful and we did not have another opportunity with these animals. There was no noticeable effect on the adult males, either on body weight or breeding activity.

The evidence suggests that the alkaloid may be toxic for certain functions such as lactation, possibly by virtue of an anti-hormone effect. It also suggests that there is individual variation in susceptibility, since 3 of the rats were able to raise, to weaning, 2 litters while consuming a test diet. This is

in contrast to the 18 litters in which all pups died.

Summary. Potato sprouts are shown to be toxic to pregnant rats when fed at a level of 10% of the diet. When the potato alkaloid solanine was incorporated into the diet at a level approximating the concentration in the sprout diet, similar effects were observed. All of the pups in 18 of 33 litters born of rats eating the test diet died before reaching weaning age. Only one of 11 control litters was lost.

1. Kline, B. F., von Elbe, H., Birdsall, J. J., *Report to Research and Development Division,* Office of Surgeon General, Dept. of the Army, U. S., Nov., 1960.

2. Muenscher, W. C., *Poisonous Plants of the United States,* Macmillan Co., New York, 1949, p200.

3. von Oettingen, W. F., *Poisoning,* W. B. Saunders Co., Philadephia, 2nd Ed., 1958, p539.

4. Willimot, S. G., *Analyst,* 1933, v58, 431.

5. Smerha, J., *Sbornik Ceskoslav., Akad. Zemedelske,* 1948, v20, 177; *Chem. Abstr.,* 1959, v43, 8538c.

6. Prelog, V., Jeger, O., in *The Alkaloids,* by Manske, R. H. F., Holmes, H. L., *Academic Press, Inc., New York,* 1953, v3, p251.

Received April 19, 1961. P.S.E.B.M., 1961, v107.

Effect of Nephrectomy and Splanchnicectomy on Plasma Disappearance of Labeled Insulin in the Rabbit.* (26763)

DAVID L. PALMER AND ROBERT E. BOLINGER
(Introduced by Sloan J. Wilson)
Department of Medicine, University of Kansas School of Medicine, Kansas City, Kan.

Studies have revealed that the disappearance rate of insulin-I^{131} from the plasma is influenced by a number of factors: plasma binding(1), state of hepatic and renal function(2,3), route and rate of administration (4), size of the endogenous insulin pool(5), and previous administration of heterologous insulin(6). Determinations based on accumulation of isotope in the liver and kidney after injection of insulin-I^{131} have implicated these organs in the process of insulin degradation(2). Such evidence is open to question since the concentration of radioactivity in these organs does not necessarily have any direct bearing on concentration of insulin. Berson *et al.*(1) and Scott *et al.*(7) have noted that the labeled material disappears from the plasma according to a curve with at least 2 different time components. On the basis of electrophoretic studies these authors have reasoned that the initial fast component of the disappearance is most representative of true insulin while the second slower component may represent the disappearance of some labeled product other than insulin. Bolinger and Slinker(3) studied the parameters of the slower component of the disappearance curve in rabbits and showed that the status of hepatic and renal function affected this component. The following study is designed to study the characteristics of the rapid component of the disappearance curve of labeled insulin in the rabbit and if possible arrive at a quantitative measure of the importance of the liver and kidney in this process. An attempt is made to define the system more specifically by including values obtained for biologic assay of insulin activity in the plasma, in order to arrive at a better estimate of the endogenous insulin pool.

Methods. Adult white rabbits were fasted for 48 hours. After light anesthetization with pentabarbital blood samples were drawn and I^{131} labeled insulin (.06 Unit) was injected intravenously. Blood samples were drawn by cardiac puncture at 10, 20, 30, 45, and 60 minutes. Plasma glucose was determined by the glucose oxidase method(8). Radioactivity was determined on both trichloracetic acid precipitates and supernatants of the plasmas, using a well counter and expressed as fraction of dose injected. Electrophoresis

* This project supported by USPHS grant-in-aid.