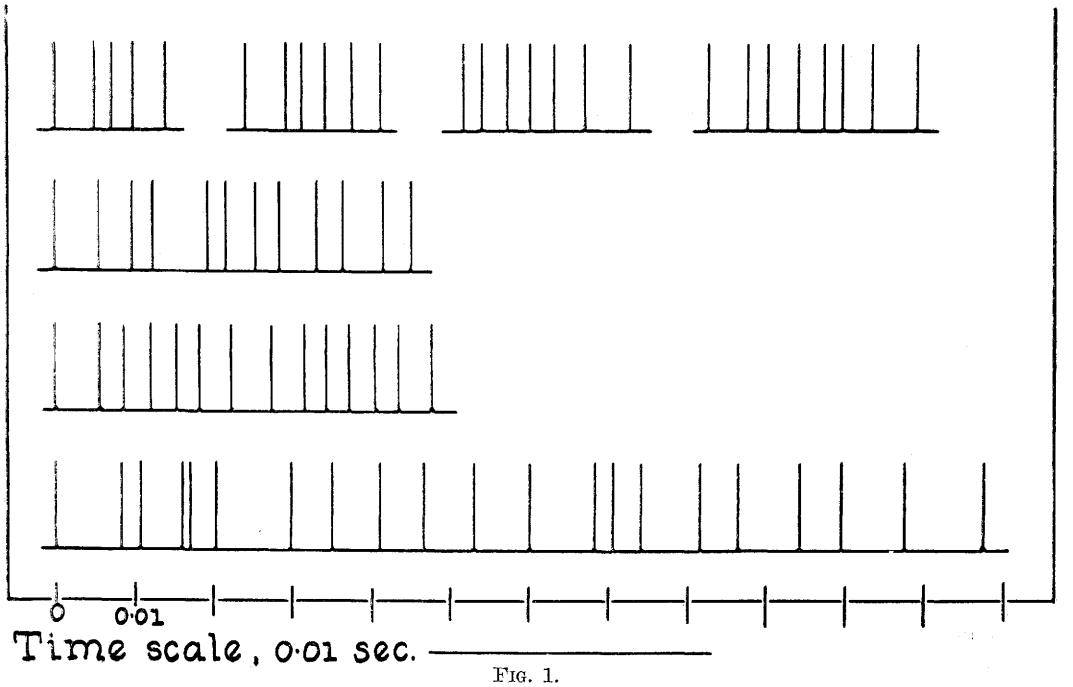

FIG. 1.

Diagram showing the spike frequency and the duration of 7 typical grouped discharges produced by D.F.P. and recorded from an anterior root of the cat. The bursts diagrammed contain from 5 to 21 spikes. Bursts consisting of a single spike, which also occurred, have not been indicated.

the twitching usually appeared first in the limb into which the drug had been injected. When recording electrodes were applied to the motor nerve roots within the spinal canal under these conditions, antidromic activity was recorded. The activity consisted of repeated bursts of nerve impulses, the impulses occurring in groups of 1 to 20, at frequencies up to 200/second during the bursts. The activity appeared to be identical with that previously reported in connection with the administration of neostigmine. (Fig. 1).

These experiments provide further evidence of the similarity of action of eserine, neostigmine, and D.F.P., and support the view that the effect of these drugs on the neuromuscular system is a result of their common action as cholinesterase inhibitors.

16152

Galactose Excretion in Young and Hepatoma Rats Fed Skim Milk Diets.*

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Adult rats receiving liquid skim milk excrete as high as 35% of the ingested galactose in the urine. When whole milk or skim milk supplemented with 3 or 4% fat is fed, the urinary loss of galactose is greatly reduced.[1] Fat increases the utilization of galactose by

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by grants from the Evaporated Milk Association, Chicago, and the National Dairy Council, Chicago, in behalf of the American Dairy Association.

[1] Schantz, E. J., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1938, **122**, 381.

the rat when either lactose or galactose is ingested in milk or synthetic type rations. The percent of the ingested galactose lost in the urine is independent of the actual amount of galactose ingested but is dependent upon the per cent of galactose in the ration.[2,3] The rate of intestinal absorption of galactose varies inversely with the percentage of fat in the diet.[3]

Heretofore, in the galactose excretion studies, adult rats were employed. This report presents results of tests with weanling rats and rats with hepatoma.

Weanling male albino rats of the Sprague-Dawley strain were kept in individual wire mesh metabolism cages. Urine samples were collected under toluene for 24-hour periods. Once or twice weekly the galactose content was determined by using a slight modification of the Shaffer-Hartmann method[4] with a factor of 1.22 to convert the values to galactose. Numerous urine samples were inoculated with *Saccharomyces cerevisiae* to test for fermentable reducing substances and in no case did this cause the amount of reducing substances to decrease.

The diet of the animals was prepared fresh daily by incorporating 10 g of commercial skim milk powder in water to a volume of 100 cc. To each 100 cc were added 12 mg of ferric pyrophosphate (Mallinckrodt N. F. VII), 0.6 mg of $CuSO_4 \cdot 5H_2O$ and 0.6 mg of $MnSO_4 \cdot 4H_2O$. The liquid diet contained 4.65% lactose of 2.45% galactose. The fat soluble vitamins were supplied as a corn oil concentrate, 2 drops per week furnishing 1.568 mg of α-tocopherol, 0.147 mg of 2-Me-1,4-naphthoquinone, 0.35 mg of β-carotene,† and 0.0098 mg of calciferol.‡

Four duplicate experiments, consisting of 6 rats each, were set up at different intervals. The average weekly galactose excretion values obtained with each group are given inTable I

The studies were repeated on rats with hepatomas,§ induced by p-dimethylaminoazobenzene or m'-methyl-p-diaminoazobenzene.[5] The hepatomas were ascertained by palpation. The results of these tests are presented in Table II.

Discussion. The urinary excretion of galactose in the weanling rat was lower than that of the adult animal on the skim milk diet. The excretion values (Table I) increased during the fifth and sixth week until the rate of excretion of the adult rat was reached. During the first few days on the liquid diet slight weight losses were observed in each group of rats, but thereafter slow growth occurred. Diarrhea was surprisingly absent in most of the young animals. When present, the excretion value was omitted due to unavoidable contamination of the urine sample. After 3 or 4 weeks on the experimental diet, 55% of the young rats had developed an opacity of the lens. This incidence was surprisingly high. Krewson *et al.*[6] had reported that the lactose content of skim milk alone was not great enough to produce cataracts. Their work differed from this in that their rats were beyond the weanling stage, weighing 80 to 100 g.

Adult hepatoma rats fed the skim milk diet showed excretions of galactose varying from zero to 1/5 the amount excreted by normal adult rats. A slow increase in excretion was noted in Rats 1 and 5 from Groups 2 and 3 respectively. Autopsy of these 2 animals showed no evidence of hepatoma. The high mortality of the tumor animals tended to obscure the results and prevent the completion of most of the experimental runs.

The lower excretion in the weanling and tumor rats approximates that obtained when 3 or 4% of fat is incorporated in the milk.

[2] Geyer, R. P., Boutwell, R. K., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1945, **162**, 251.

[3] Nieft, M. L., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1947, **167**, 521.

[4] Osgood, E. E., *A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students*, Philadelphia, 1935, 301.

† 90% β-carotene and 10% α-carotene.

‡ Crystalline irradiated ergosterol.

§ The hepatoma animals were obtained through the courtesy of Dr. C. A. Baumann and coworkers.

[5] Rusch, H. P., Baumann, C. A., Miller, J. A., and Kline, B. E., *A. A. A. S. Research Conference on Cancer*, 1944, 267.

[6] Krewson, C. F., Schantz, E. J., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 573.

TABLE I.
Galactose Excretion by Male Weanling Rats.

Group	Weekly galactose excretion values* (% of galactose ingested) 1	2	3	4	5	6	7	8	9	10	No. of animals with lens opacity
1	5.69	8.66	8.60	9.7	12.02	—	—	21.7	25.1	—	5
2	7.41	6.46	5.03	3.6	12.20	20.5	20.9	27.7	—	—	2
3	—	6.90	4.45	10.3	11.40	20.1	19.1	32.0	40.0	31.0	3
4	4.61	4.61	8.35	11.56	14.40	16.77	19.8	29.1	—	—	3

* Each group consisted of 6 animals, but contamination due to diarrhea sometimes occurred. Because the contaminated samples were omitted, each figure in the table represents the average urinary galactose excretion of 3 to 6 animals.

TABLE II.
Galactose Excretion by Male Rats with Hepatoma, Fed Skim Milk.

Group	Rat	Weekly galactose excretion values* (% of galactose ingested) 1	2	3	4	5	6	7	8	9	10
I	1	5.05	5.05	Dead							
	2	2.98	3.38	6.49	Dead						
	3	2.98	Dead								
	4	0.0	4.3	3.38	5.37	4.78	2.39				
	5	—	—	5.97	8.96	9.23	Dead				
	6	—	—	0.0	3.78	4.78	2.98				
II	1	5.97	6.75	12.30	17.6	24.6	26.0				
	2	6.73	2.58	Dead							
	3	5.69	5.15	4.50	Dead						
III	1	5.97	—	—	0.0	—	—	3.78	5.97	Dead	
	2	6.97	—	—	0.0	—	—	5.97	0.0	Dead	
	3	9.25	—	—	2.98	—	—	6.75	Dead		
	4	4.55	—	—	1.79	—	—	4.30	2.98	4.08	5.97
	5	3.38	—	—	1.79	—	—	20.01	25.40	31.00	33.40
IV	1	4.80	9.4	Dead							
	2	2.92	Dead								

* Each figure represents the galactose excretion value of one animal.

In the young animal more efficient utilization of galactose may be necessary for the more adequate utilization of the high lactose diet during early life. Evidence that the system involved may be one present in the liver is substantiated by the results obtained in rats with hepatomas.

Kosterlitz[7] and others have shown that an increased amount of glucose is found in the liver during galactose assimilation. Greenstein[8] reports that rat hepatoma possesses an acid phosphatase activity double that of normal liver and an alkaline phosphatase activity 120 times as great. This may explain the decreased galactose excretion in the tumor animal. A similarly increased phosphatase system may occur in the young animal, and induce a greater conversion of galactose-1-phosphate to glucose or glycogen. In the older, more mature animal the lactose intake is smaller and the galactose utilization system must compete to a lesser degree. The fact that the adult liver can convert galactose at a certain rate forms the present basis for the galactose tolerance test as a measure of liver function. This study would seem to indicate that the test may be inadequate in determining dysfunction due to tumor.

The hepatomas were ascertained to be originally present through palpation. However, in 2 observations autopsy failed to show

[7] Kosterlitz, H. W., *Biochem. J.*, 1943, **37**, 181.

[8] Greenstein, J. P., *J. Nat. Cancer Inst.*, 1942, **2**, 511.

any evidence of liver tumor. Whether coincidental or not, the influence of the liquid skim milk diet on the resolving of hepatomas and other related disturbances is worthy of investigation.

Conclusions. The urinary excretion of galactose in the young rat is lower than that of the adult animal when a supplemented skim milk diet is fed.

On such a diet, adult rats, with hepatoma induced by azo dyes, excrete a lower percentage of galactose in the urine than do normal adult rats.

16153

A Method for Producing Sustained High Penicillin Levels in the Blood.

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(Introduced by Gregory Shwartzman.)

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Improvement in antibiotic therapy may be expected if high penicillin levels could be maintained in the blood stream and tissues for prolonged periods. The required period of therapy may perhaps thereby be shortened and infections due to exceptionally resistant organisms may more readily be brought under control. Furthermore, high, sustained penicillin blood levels may result in tissue saturation and greater penetration of the drug into foci of infection.[1]

To date, efforts to achieve prolonged levels consisted in the employment of two methods, (a) the use of oil, wax and other menstrua to delay absorption of penicillin introduced into a local depot and (b) the use of drugs capable of delaying the ordinarily rapid excretion of penicillin through the kidneys, *i.e.* para-aminohippuric acid,[2] and caronamide.[3] Caronamide (4'-carboxyphenylmethanesulphonanilide) has been shown by Beyer[4] to have an inhibitory effect on the renal tubular excretion of penicillin. In addition, attempts have been made to obtain high levels by continuous intravenous drip and by the periodic administration of large "booster" doses of the antibiotic. The dose and speed of injection have been limited under the continuous intravenous method because of thrombo-phlebitis.

In this study, we have achieved sustained and unusually high levels of penicillin in the blood stream by the periodic intravenous injection of large doses of crystalline penicillin* when caronamide† was used as an adjuvant to block rapid excretion through the kidney.

Initially it was planned to obtain high intracardiac levels of penicillin by introducing the drug through an intracardiac catheter passed into the right ventricle or pulmonary artery. Preliminary experiments on dogs demonstrated, however, that the levels‡ in the aorta achieved by rapid injection of equally large doses of penicillin in concen-

1 Gerber, I. E., Shwartzman, G., and Baehr, G., *J. A. M. A.*, 1946, **130**, 761.

2 Beyer, K. H., Flippin, H. F., Verwey, W. F., and Woodward, R., *J. A. M. A.*, 1944, **126**, 1007.

3 Crosson, J. W., Boger, W. P., Shaw, C. C., and Miller, A. K., *J. A. M. A.*, 1947, **134**, 1528.

4 Beyer, K. H., *Science*, 1947, **105**, 94.

* Sodium and calcium salts of crystalline penicillin kindly supplied by Schenley Laboratories, Inc.

† Kindly supplied by Sharp and Dohme, Inc.

‡ The method of penicillin assay employed is a broth tube dilution method using *Staphylococcus aureus* H as the test organism and fresh meat extract broth as the medium. The minimal concentration of the standard penicillin required to inhibit the inoculum of 5 x 10^2 *Staphylococcus aureus* H cells was 0.02 units per ml. The standard penicillin was obtained from the U. S. Department of Agriculture. All titrations of serum levels were accompanied by and compared with this standard.