

Accordingly, bile was returned every hour in 3 dogs for 6 days. Fat absorption during the last 5 days was within normal limits⁷ in one dog and considerably improved in the others. Since the plotted points (open circles) are well below the regression line in Fig. 1, the improvement appeared to be due largely to the continuous presence of bile, and not to the increased quantity of cholic acid secreted and returned. To check this point further, bile was returned every 4 hours in the same 3 dogs. The quantity of cholic acid returned did not change, but fecal fat excretion increased so that the plotted values (crosses) fell near the original regression line.

Finally, fecal fat excretion was measured in 3 dogs after cessation of bile return, in order to determine the rapidity with which the fat absorptive defect develops when bile is excluded. From the third through the seventh day after bile return was stopped the fecal fat excretion averaged 17.8 g per day; from the eighth through the thirteenth day, 22.0 g per day. The former value is significantly less, by group comparison analysis,⁸ than the mean fecal fat excretion obtained in the present and previous⁷ study during 5-day periods begun 10 days or more after the operation. The results indicate that impaired fat absorption develops rapidly when the bile is first excluded, then more slowly to a maximum after about 7 days. The presence of bile acids in the scraped intestinal mucosa of dogs 24 hours, but not 1 month after bile exclusion,⁹ suggests a possible reason for the de-

lay in development noted.

Discussion. The present results demonstrate that previous failure to correct steatorrhea in bile fistula dogs by feeding them whole ox-bile containing 6 g of cholic acid with the single daily meal was probably due to the failure of this procedure to provide adequate amounts of the bile in the intestine over the period required to complete fat absorption. Although continuous hourly administration of bile was employed successfully in this study to restore normal fat absorption, this does not imply that hourly return must be continued *throughout a 24-hour day* to complete the absorption of a single daily fat meal. However, the establishment of the fact that bile must be present in the intestine continuously for at least most of the day now makes it possible to investigate further the bile component(s) and dosages necessary for normal fat absorption, and to assay bile replacement measures.

Summary. The steatorrhea of 3 bile fistula dogs given a fatty meal once daily was nearly completely corrected when the animals' own bile was returned to the duodenum every hour. The steatorrhea persisted, however, when bile was returned every 4 or 8 hours. Since the total quantity of cholic acid returned during the 1- and 4-hour regimes was the same, the importance of continuous presence, apart from total daily quantity present, in the intestine is demonstrated.

⁹ Verzar, F., and Von Kuthy, A., *Biochem. Z.*, 1931, **230**, 451.

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17163. Observations on Experimental Dental Caries. XI. Influence of Pregnancy.

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The results of the clinical investigations on the relationship between pregnancy and dental caries have been contradictory. However, most of the controlled studies made in the last years negate the validity of the old popu-

lar idea that pregnancy increases the susceptibility to dental caries. Day,¹ for example,

¹ Day, C. D. M., *Indian J. Med. Research*, 1947, **35**, 101.

in a recent study carried out with a large number of subjects who had gone through various numbers of pregnancies, and who were divided into several age groups, found no significant differences which could support the statement of a relationship between pregnancy and dental caries incidence. Furthermore, Ziskin and Hotelling,² also in clinical studies, found evidence to show that pregnancy not only does not increase dental caries but that factors operating during pregnancy actually prevent tooth decay to a significant degree.

In hamsters, we³ found that pregnancy and lactation do not influence caries activity in any direction. Considering Ziskin and Hotelling's findings, we have studied in the present experiment the influence of pregnancy alone, in order to test the possibility that a significant decrease of dental caries activity brought about by pregnancy alone, might be annulled by a harmful effect of lactation.

Experimental. Thirty female hamsters, 23 days old, from litters of a colony maintained on Purina Laboratory Chow* and raw milk, were littermate distributed into two groups as follows: Group I, 17 females, and Group II, 13 females. Both groups were reared for 100 days on the following diet of sub-optimal nutritional value: finely ground yellow corn 25%, sucrose 25%, corn starch 20%, powdered whole milk 22%, ether-extracted yeast 5%, alfalfa meal 2%, salt mixture† 1%, and d,l- α -tocopherol acetate 0.010%. The ration and tap water were available *ad libitum*.

Both groups were kept during the first 56 experimental days in screen bottom cages without bedding. At this time the 2 groups were transferred to breeding cages containing sawdust on the bottom, and excelsior for bedding; thereafter 9 sexually mature males kept on the stock colony diet were mated for 13 days with the 17 females of Group I.

² Ziskin, D. E., and Hotelling, H., *J. D. Res.*, 1937, **16**, 507.

³ Granados, H., Glavind, J., and Dam, H., *Odontologisk Tidskrift*, 1948, **56**, 388.

* From Ralston Purina Co., St. Louis, Mo.

† The salt mixture used was McCollum's Salt Mixture No. 185, supplemented with 13.5 mg KI, 139 mg CuSO₄, 5H₂O, and 556 mg MnSO₄, 4H₂O per 100 g.

TABLE I.
Caries Activity in the Two Groups.

	Group I (pregnant) 14 ♀ ♀	Group II (nonpregnant) 13 ♀ ♀
% of animals affected	100	100
Avg No. of carious molars	8.0	8.5
Avg No. of carious lesions	11.5	10.8
Avg caries scores	6.0	6.2

Every litterborn from Group I was removed from the mother within 6 hours after parturition. Both groups remained in the breeding cages until the end of the experiment.

On completion of the 100 day experimental period the animals were sacrificed and autopsied. The molars were prepared for examination in the usual way,⁴ and the carious lesions were recorded and scored.⁵

Results. In group I out of the 17 mated females 2 died during labor, and one had sterile mating. Therefore, only 14 females, those who had normal parturitions, finely formed Group I.

Table I shows the caries activity of the two groups. It is apparent that essentially the same incidence and extent of carious lesions were found in both groups. Therefore, under the conditions of this experiment, pregnancy did not influence in any direction the caries activity. Thus can be stated that neither pregnancy and lactation,³ nor pregnancy alone influence in any direction the caries susceptibility of the Syrian hamster. These experimental findings agree with the results of most of the controlled clinical studies carried out in the last years¹ on this subject.

It is generally recognized that pregnancy and lactation are physiological states of increased nutritional stress for the mother, since she has to face the double task of supplying proper amounts of nutrients necessary for the offspring as well as her own metabolism. Certain workers⁶ have stated that the increased caries activity during pregnancy reported by them has been due to

⁴ Granados, H., Glavind, J., and Dam, H., *Acta Pathol. et Microbiol. Scandnav.*, 1948, **25**, 453.

⁵ Keyes, P. H., *J. D. Res.*, 1944, **23**, 439.

⁶ Wohl, M. G., Dietotherapy. Clinical application of modern nutrition, W. B. Saunders Co., Philadelphia, 1945, p. 540.

quantitative and/or qualitative deficiencies in the diet. In our previous experiment on the influence of pregnancy and lactation³ on caries activity, as well as in the present study, we used a sub-optimal diet in order to sharpen the nutritional deficiency of the group undergoing pregnancy, and thus obtain sharper differences of caries activity between the two groups in case of any relationship between dietary deficiencies and dental caries.

However, as it can be seen from the data presented, neither pregnancy and lactation³ nor pregnancy alone influenced caries activity

in any direction. These facts are in open contradiction with the alleged increase of caries activity as a consequence of dietary deficiencies during pregnancy.

Summary. The influence of pregnancy on caries activity has been studied in hamsters reared from weaning for 100 days on a sub-optimal diet, with their respective litter-mate controls. The results show that pregnancy does not influence caries activity in any direction.

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17164. Evidence for a Steroid Compound in Cane Juice Possessing Antistiffness Activity.

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The isolation from crude cane molasses and crude unheated cane juice of a fraction capable of alleviating an induced wrist stiffness in guinea pigs raised on a skim milk diet, was described by van Wagtendonk and Wulzen.¹ Later investigations by Oleson, *et al.*,² and Petering and coworkers³ indicate that ergostanyl acetate and α -ergostanyl acetate are effective curative agents. We are therefore prompted to report that an active fraction of steroid nature has been isolated from the original cane juice fraction, and in our assay methods has proved to be a curative agent in dosages of at least 0.01 γ .

Experimental. A product obtained from the cane juice and melting at 81-82°¹ did not prove to be homogeneous when subjected to carbon-hydrogen analyses. It was therefore subjected to various procedures for further

purification. Chromatic adsorption on columns of magnesium oxide or activated alumina failed to yield pure and homogeneous products. Repeated recrystallization from alcohol, followed by recrystallizations from acetone and cyclohexane yielded a compound melting at 164-166° that was homogeneous according to the carbon and hydrogen analyses and highly active in curing the induced stiffness in deficient guinea pigs.

a. Activity tests. The assay for activity was carried out according to the previously described methods.^{1,4} The compound was dissolved in Wesson oil and administered orally to guinea pigs that had been kept on the deficient regime of skim milk powder to which the necessary vitamins and minerals had

TABLE I.

Therapeutic Test of High Melting Compound Carried Out on Guinea Pigs on Skim Milk Ration.

Level of assay in γ (5 times)	No. of animals	Cured	No cure
1	3	3	0
0.01	27	23	4

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¹ van Wagtendonk, W. J., and Wulzen, R., *J. Biol. Chem.*, 1946, **164**, 597.

² Oleson, J. J., van Donk, E. C., Bernstein, S., Dorfman, L., and Subbarow, Y., *J. Biol. Chem.*, 1947, **171**, 1.

³ Petering, H. G., Stubberfield, L., and Delor, R. A., *Arch. Biochem.*, 1948, **18**, 487.

⁴ van Wagtendonk, W. J., and Wulzen, E., *Arch. Biochem.*, 1943, **1**, 373.