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Fate of Food Introduced Directly into the Fetal Stomach.*

J. Francis Hartmann and L. J. Wells.

From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.

The literature on the human fetus contains records of several studies devoted to the time of origin of function in the digestive system. Thus, the presence of gastric glands at the 4th and 5th month has been reported[1,2] and various enzymes have been identified at about the 4th month.[3–6] From such observations as these, it has been inferred that the fetus is prepared to carry on various digestive processes in advance of birth. Also, as is well known, premature infants may survive. It is, however, an entirely different question as to whether the fetus can actually digest food during the time when its nutrition is being supplied via the placenta.

Recently, methods of experimenting directly upon fetal rats have been devised. These methods involve subjecting fetuses to surgical procedures and subsequently returning them to the maternal abdominal cavity for varying periods of time, the placental circulation being maintained intact throughout.[7]

As a means of securing direct evidence concerning antenatal digestive abilities, these operative procedures were utilized in the present series of exploratory experiments.† A total of 73 rat fetuses, with placental circulation kept intact, were subjected to laparotomy and subsequent return to the maternal abdominal cavity.[8] Of these, 48 lived to autopsy, giving an overall survival value of approximately 66%. The fetuses were divided into three dietary series.

In the first group (Table I), raw beef or venison, in amounts ranging from 1.0 to 3.5 mg, was inserted directly into the exteriorized fetal stomach through a minute perforation in the stomach wall. The latter was then closed with a single stitch, the fetal abdominal wall was sutured, and the fetus returned to the maternal abdominal cavity. At autopsy, the stomach contents were examined, and muscle fibers were found to be microscopically recognizable as late as 53 hours after operation. It will be noted in Table I that in the 2 fetuses examined at 47 hours, the presence of muscle fibers at autopsy was doubtful. This is due to the fact that in these 2 instances, a microscopic analysis of the stomach contents was not carried out. In all cases, however, there was a discrete mass present in the stomach, representing the remains of the meat originally introduced. The mass was somewhat softer in consistency than when introduced, and upon teasing it under the microscope, the changed consistency was found to be due to a softening of the connective tissue elements. The muscle fibers themselves gave no morphological evidence of having been attacked by digestive enzymes. Ligation of the fetal esophagus did not seem to influence the results.

In the second series (Table II), cow's milk or human milk was injected directly into the exteriorized stomach. The first group represents fetuses returned to the maternal abdominal cavity after injection, the second group

* Aided by grants from Ciba Pharmaceutical Products, Inc., and from the Graduate Research Fund of the University of Minnesota.

1 Kolliker, A., *Entwicklungsgeschichte des Menschen und der höheren Thiere,* Wilhelm Engelmann, Leipzig, 1879.

2 Scott, G. H., *Am. J. Dis. Child.,* 1925, **30**, 147.

3 Ibrahim, J., *Biochem. Z.,* 1909, **22**, 24.

4 Keene, M. F. L., and Hewer, E. E., *Lancet,* 1929, **1**, 767.

5 Tachibana, T., *Jap. J. Obst. and Gynec.,* 1927, **10**, 27.

6 Tachibana, T., *Jap. J. Obst. and Gynec.,* 1928, **11**, 92.

7 Wells, L. J., *Anat. Rec.,* 1946, **94**, 530.

† The observations reported here have been abstracted,[8] and it seems desirable to present the data in fuller detail since it is unlikely that additional new information could be obtained without first modifying the experimental approach.

8 Hartmann, J. F., and Wells, L. J., *Anat. Rec.,* 1948, **100**, 673.

TABLE I.
Introduction of Meat into Fetal Stomach.

Hrs in stomach	No. of fetuses	Exp. begun, prepartum age in days	Amt, mg	Muscle fibers present at autopsy
6	1	22	3.5	+
6	1	22	3.2	+
23	1	22	3.0	+
23	1	22	1.5	+
47	2*	20	1.4	±
49	1*	20	0.8	+
52	1	20	1.8	+
53	1	20	1.4	+

* Esophagus ligated.

TABLE II.
Injection of Milk into Stomach.

Hrs in stomach	No. of fetuses	Exp. begun prepartum age in days	Amt. injected, cc		Stomach contents at autopsy
			Cow's milk	Human milk	
		A. Fetuses.			
¼	1	22	.05		Flocculent curd
¼	1	22	.03		" "
3	1	22	.05		Soft discrete curd
7	1	22	.05		" " "
7	1	22	.05		Hard curd
23	1*	21	.05		Flocculent curd
27	1	21	.045		Whitish mucoid mass
29	1*	21	.04		Trace of flocculent curd
49	1	20	.025		Flocculent curd
52	1	20		.05	Whitish mucoid mass
52	1	20		.05	Flocculent curd
52	1	20		.03	" "
53	1	20		.02	Whitish mucoid mass
		B. Caesarian Newborns.			
½	2	22	.05		Fluid
¾	1	22	.03		Flocculent curd
¾	1	22	.05		Fluid
¾	1	22	.05		Soft curd
2	1	22	.03		Firm "
2	1	22	.05		Hard "
7	1	22	.05		Fluid
15	2	22	.05		Hard curd
19	1	22	.10		Soft "
23	1	22	.10		Curd firm at cardia
30	1	22	.075		Soft curd

* Esophagus ligated.

comprises Caesarian newborns taken on the last day of gestation and kept at room temperature for the lengths of time indicated in the first column. At autopsy, the stomachs of fetuses examined shortly after operation contained flocculent curds. Curds found 7 hours postoperatively were soft to hard, those examined at 23 hours being again flocculent in character. Traces of flocculent curd, however, were still present in the cardiac region of the stomach at 49 and 52 hours after injection. From observation of the stomach contents in these animals, one gains the impression that curds begin to form soon after the milk is injected, that they later solidify, and still later soften again. The softening is more noticeable at the pyloric end of the stomach. Whether or not this indicates an actual progression of digestive activity in the fetal stomach is not entirely clear from the present data, and chemical analyses of the stomach contents will undoubtedly supply additional evidence on this point. In some instances, no discrete curd was found at

TABLE III.
Normal Newborns Allowed to Nurse.

Hr in stomach	No. of animals	Stomach contents at autopsy
13	1*	Empty; probably had not nursed
15	1*	Soft curd
16	1	" "
21	1	" "
27	1	Trace of curd
37	1	Empty

* Kept at 37°C.

TABLE IV.
Injection of 10% Uncooked Corn Starch into Fetal Stomach.

Hrs in stomach	No. of fetuses	Exp. begun prepartum age in days	Amt in cc	Iodine test at autopsy			
				Rectum	Ileum	Duodenum	Stomach
21	1*	22	.05			+	+
22	1*	22	.05			+	+
23	1*	22	.05	—		+	+
24	1*	22	.05	—		+	+
24	1*	22	.01		+	+	+
27	1	21	.02	—		+	+
27	1	21	.02	—		—	—
27	1	21	.01	—		—	—
30	1	21	.01	—		—	—
30	1	21	.05	—		—	—
49	2	20	.015	—		—	—

* Caesarian newborns.

autopsy, but instead the stomach contained an abundance of mucoid material in which the milk was dispersed, so that no definite curd was observable. The mucoid mass probably represents maternal peritoneal fluid swallowed by the fetus and partially coagulated in the stomach, after being mixed with mucus of fetal origin.

The emptying time of the stomach in operated animals was unexpectedly long,[9] and the delay in emptying is no doubt due in part to operative trauma. In an attempt to evaluate the importance of trauma in delaying the emptying time, a number of normal newborns were allowed to nurse, then separated from the mother, and autopsied from 13 to 37 hours later (Table III). Traces of ingested milk were found in the stomach at 27 hours in one animal, but not in the one examined at 37 hours. The curds were in all cases softer than the hardest curds formed from either cow's milk or human milk. It appears probable that under normal postnatal conditions, the supply of milk in the stomach is constantly replenished by nursing, so that complete emptying never actually takes place. However, the fact that the stomach of the normal newborn rat does not empty completely until after 27 hours, plus the fact that the fetuses injected on the 20th prepartum day were in an earlier stage of development than the newborns, leads to the belief that operative trauma is not the sole cause of the delay in emptying time observed in the fetuses.

The third dietary series (Table IV) received varying amounts of a 10% suspension of uncooked corn starch, administered in the same manner as the milk. At autopsy, the stomach and parts of the intestine were removed to separate glass slides, treated with iodine, and examined under the microscope for the presence of starch grains. Up to 27 hours after injection, blue-stained starch grains were found in the stomach and small intestine, but after 27 hours, none were detectable by the iodine test. The possibility suggests itself that digestion had proceeded at least to the stage of achroodextrins. Since, however, the conditions of the experiment did not preclude bacterial digestion, this remains only a possi-

9 Windle, W. F., and Bishop, C. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 2.

bility, pending more elaborate tests of the gastrointestinal contents to show whether or not the disappearance of the starch grains is attributable to the action of amylolytic enzymes of the fetus itself.

In conclusion, the data show that in the fetal rat with the placenta still functioning, the stomach empties more slowly than might have been anticipated, and they suggest that the fetus has the ability to digest in part such introduced foods as milk and starch. It would seem that the ability to digest meat is less well developed or absent. This exploratory study demonstrates the feasibility of obtaining additional direct experimental data on the digestive abilities of the fetus.

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Carcinogenic Effect of Sulfonamides.*

ALF T. HAEREM. (Introduced by G. F. Dick.)
From the Department of Medicine, University of Chicago.

Early workers in the field appreciated the possibility of sulfonamide carcinogenesis because of the similarity in structural formulae between the sulfonamides and known carcinogenic agents of the aniline dye group. Marshall suggested investigation of the possible carcinogenic effects of sulfonamides and Lewis[1] subsequently reported that the injection of sulfanilamide in olive oil was not followed by the appearance of tumors in the strain of mice used. Similar results were later reported when other workers repeated these experiments.[2] Using a slightly different strain of mice and material it was later reported[3] that spindle cell sarcomata developed at the site of injection of 15 mg of sulfanilamide in lard in a small percentage of Swiss albino mice so injected. Since that report the entire experiment has been repeated. Using 20 mice of the same strain, under the same conditions, the same amount of sulfanilamide was injected. Eight (40%) of the group developed a spindle cell sarcoma at the injection site. The mice developing tumors survived from 54 to 269 days (average was 145 days after injection). A similar number of mice injected with lard alone developed no tumors.

In an attempt to further evaluate the sulfonamides in regard to carcinogenic properties, these studies were repeated on albino rats. One group was injected with sulfanilamide in lard and this was compared with a group injected with a known carcinogenic agent (1:2:5:6 dibenzanthracene) in lard. Controls consisted of one group in which lard alone was injected and one group in which no injections were made. Similar observations were made on rats injected with sulfapyridine.

Group I. Sulfanilamide injections (test animals). Two injections of 0.015 g sulfanilamide were given to each of 10 rats. The first injection was made at the age of 14 weeks into the left groin and the second at 23 weeks into the right groin. Nine months after the first injection one of the rats had developed a huge mass in the left groin. Biopsy revealed the tumor to be a spindle cell sarcoma. The tumor was readily transplanted to another rat. Autopsy one month later on the original rat revealed a frankly invasive tumor which had metastasized to both lungs. The cell structure of the original tumor and metastases was identical. Both were composed almost entirely of spindle cells. Mitotic figures were

* Aided by a grant from the Fenger Fund.

1 Lewis, M. R., *Am. J. Cancer,* 1938, **34**, 431.

2 Zamecnik, P. C., and Koletsky, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 391.

3 Haerem, A. T., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 536.