Conclusion. Rats fed a diet containing casein have a considerably higher resistance to enteritidis infections (I.P.) than controls fed either wheat gluten or soy bean flour.

8984 C

A Biochemical Effect of Ether on the Gut.

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Physiological techniques have demonstrated[1] a marked inhibition of the gut during ether anesthesia and for a short time during recovery. In other cases of reduced motility of the gut, as in atonic constipation, complementary regular changes in putrefaction have been noted. Difficulty has been encountered in showing any such regular changes in the excretion of putrefactive bodies in the urine of anesthetized subjects.[2] It was felt that more distinct changes could be noted by examining the fecal material directly.

Bergeim[3] introduced a technique for determining the putrescibility of proteins *in vivo*, which depends essentially on observation of the stronger reduction processes occurring in the gut contents. This test may equally well be used to note changes in putrefaction of a single protein due to physiological factors such as motility of the gut. When so used, it is not subject to certain criticisms[4] of the original method, since the diet is constant. Two of Bergeim's[3] protein diets* were used in the following experiments: casein, a protein of low putrescibility, and egg albumin, which is more highly putrescible. Because of the extended period the rats were fed these diets, 2% of Wesson's[5] salt mixture were added.

Reduction processes in the gut associated with putrefaction[3] may be estimated by noting the per cent reduction of ingested ferric oxide incorporated in the Bergeim diets. Feces containing the Fe^{++}-Fe^{+++} mixture are heated at 100°C. for 10 minutes with dilute HCl, and a

1 Miller, G. H., *J. Pharm. Exp. Therap.*, 1926, **27**, 41.

2 Killian, H., *Narkose zu operativen Zwecken*, Berlin, 1934.

3 Bergeim, O., *J. Biol. Chem.*, 1924, **62**, 45, 49.

4 Hoelzel, F., *J. Biol. Chem.*, 1929, **83**, 331.

* White dextrin, 800 gm.; casein or egg albumin, 200 gm.; granulated agar, 10 gm.; ferric oxide, finely powdered, 10 gm.; salt mixture, 20 gm.

5 Wesson, L. G., *Science*, 1932, **75**, 339.

TABLE I.

Ferric Reduction in the Gut of Rats fed a Dextrin-Casein Diet. Results Expressed in % Fe^{++} of Total Iron.

Group	No. of Rats	Treatment	Ferric Reduction, Days of Diet							
			1	2	3	4	5	6	7	11
A	10	Ether, 2.5 mM/L. for one hour on 4th day	61±2	44±2	29±2	*28±2*	21±4	17±2	13±3	14±2
B	10	Similar treatment, but repeated 5th and 6th days	70±4	44±5	31±2	*27±3*	*34±3*	*27±2*	15±2	17±2
C	10	Epinephrine HCl, divided doses, 4th to 6th days, as in text	52±5	39±2	31±4	*21±3*	*23±3*	*16±2*	*18±2*	*26±3
D	10	Untreated controls	60±2	60±4	27±4	24±2	25±3	16±1	14±2	17±3
Aver.			61	47	30	—	—	—	15	16

*Large sloughs at site of injection in 2 rats; necrotic material eaten by other rats.

TABLE II.

Ferric Reduction in the Gut of Rats Fed a Dextrin-Albumin Diet. Results Expressed in % Fe^{++} of Total Iron.

Group	No. of Rats	Treatment	Ferric Reduction, Days of Diet									
			1	2	3	4	5	6	7	8	10	15†
A	10	Ether, 2.5 mM/L. for one hour, 4th to 7th days	46±3	55±2	41±3	*43±2*	*55±2**	*54±4*	*64±3*	33±4	35±2	17±3
B	9	Similar treatment, 4th to 6th days	49±4	37±2	43±4	*48±3*	*59±3*	*55±4*	37±4	36±4	34±2	16±1
C	10	Ether, middle of 4th day	46±5	42±4	40±4	*46±2*	48±3	37±4	37±3	37±2	35±4	19±3
D	9	Untreated controls	56±4	39±2	39±2	40±2	50±3*	41±4	38±2	37±5	34±2	19±4
Aver.			49	43	41	—	—	—	—	36	35	18

*One rat died in each of groups A and D; transient high reduction values resulted from other rats of these groups eating the carcasses.

†Fasted on 11th day and fed Dextrin-Casein diet 12th to 15th days.

colorimetric comparison of the Fe^{+++} content of 2 aliquots of the filtrate from this mixture is made after treating one aliquot with $KMnO_4$. The per cent reduction may thus be obtained directly, without the necessity of determining actual total Fe present. Modifications of Bergeim's[5] technique which were found to increase the accuracy of the method were: use of boiled distilled water to make up the dilute HCl; 10 minutes heating; and the use of mineral oil to exclude atmospheric oxygen during the heating.

Tables I and II illustrate changes in putrefaction brought about by repeated ether administration. Four groups of 9 or 10 rats each were used for each diet. Feces were collected by digital pressure on the rectum, 24 hours after beginning feeding of the diets and in the same way at the same time each day thereafter. Feces were immediately analyzed after collection, as above. When the groups had approached a stable point as noted by lowering of the standard deviation of the mean and agreement of results in the 4 groups, at the beginning of the 4th day, treatment was instituted in 3 of the groups while the 4th was kept as untreated controls. Of the groups receiving casein, the first was anesthetized with 2.5 mM/L. of ether in oxygen for one hour at the beginning of the 4th day, the 2nd was similarly treated on the 4th to 6th days, while the 3rd group was treated with 3 doses of 0.2 mg./kg. of epinephrine HCl given subcutaneously at 4-hour intervals on the 4th and 5th days respectively, and 4 similar doses given on the 6th day. This follows Cori's[6] suggestion for "continuous" epinephrine administration. Of the groups receiving egg albumin, the first was treated similarly with ether at the beginning of the 4th to 7th days, the 2nd on the 4th to the 6th days and the 3rd at the middle of the 4th day.

Table III represents changes in putrefaction immediately after treatment. Two groups were used of rats previously fed the casein diet until their feces showed very low reduction. One group was observed without treatment for a day, and then given a single subcutaneous dose of 0.5 mg./kg. of epinephrine HCl. The other group was treated with ether for one hour on the first day and observed without treatment on the following day. It was found difficult to collect feces from all of the treated animals at each point, but a sufficient number were obtained to yield significant results. The average number of determinations at each point excepting the 2 noted in the table as single observations was 8.6 for the control groups and 7.7 for the experimental groups.

[6] Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

TABLE III.

Course of Intestinal Reduction after Single Drastic Treatments. Groups of Ten Rats Fed Bergeim's Dextrin-Casein Diet.* Results Expressed in % Fe^{++} of Total Iron.

Group	Treatment	Before Treatment	Time in hours after start of treatment										
			1	2	3	4	5	6	8	10	12	18	24
A	Controls, untreated, 1st day	16±1	16±1	—	16±2	—	16±1	—	16±1	—	16±1	—	16±1
	Single dose 0.5 mg./Kg. Epinephrine HCl 2nd day	16±1	16±2	18±3	19±3	18±2	(14)	—	17±2	21±3	21±2	28±2	31±4
B	Ether, 2.5 mM/L. for one hour, 1st day	16±1	(21)	17±2	19±1	20±3	21±2	23±3	21±2	22±2	22±3	20±3	17±2
	Controls, untreated, 2nd day	17±2	—	16±2	—	17±2	—	—	—	16±2	—	—	17±1

*Maintained on casein diet 6 days before starting treatment.
— blank spaces indicate collection of feces without analysis; control of influence of manipulation.
( ) parentheses indicate result of a single observation only.

The results as tabulated may be compared only with values for the control groups maintained on the same diet, as it may be seen readily that the type of protein fed has far greater influence on reduction of ferric iron than does stasis. It may be noted that the usual laboratory diet for rats is highly putrescible, as shown by initial values in Tables I and II.

If the results are examined in this way, it appears that a significant variation from the controls occurs on the 3rd day of repeated ether administration, in animals fed the casein diet. Repeated treatment with epinephrine produces little effect, possibly because the gut has sufficient time to recover activity before the fecal samples are taken. With animals on the albumin diet, the standard deviation of the mean is about the same as with casein-fed rats, but the reduction is greater, so that a significant variation appears on the 2nd day of repeated etherization. In the single acute experiments of Table III, it appears that the peak of the effect of ether is passed by the 12th hour, so that greater effects would have appeared in Tables I and II if 12-hour samples had been taken in place of those at 24 hours. The marked, continued effect of 0.5 mg./kg. of epinephrine HCl is in contrast to the effect of 0.2 mg./kg., and the latency of the effect is further indication of its extent, since this latency is probably a reflection of the slower passage of feces. The closer similarity between the effects of ether and 0.5 mg./kg. of epinephrine than 0.2 mg./kg. occurs elsewhere,[7] also.

Since 24-hour urine specimens of patients anesthetized with ether or other anesthetics showed no regular changes in concentration of indican, urobilinogen, ethereal sulphates or free and conjugated phenols during 2-3 days post-anesthetically, no attempt was made to collect sufficient cases to give statistically significant results. The findings agree with those in the literature,[2] and may be explained through pre-anesthetic medication, time of anesthesia and the nature of the diet before and after anesthesia.

Summary. Ether acts to increase reduction processes in the gut of rats, and gives rise to significant changes in these processes if the anesthesia is repeated on successive days. Epinephrine HCl showed no such effect on fecal samples taken 24 hours after beginning treatment with 0.2 mg./kg. given subcutaneously at 4-hour intervals for 8 or 12 hours, but a single dose of 0.5 mg./kg. resulted in a marked increase of reduction in fecal samples taken from 10-24 hours later. Reduction processes in the rat gut after surgical anesthesia with ether for one hour reach a peak with the fecal sample

[7] Emerson, G. A., *J. Pharm. Exp. Therap.*, 1935, **55**, 90.

taken 6 hours after induction of anesthesia, and show some augmentation over those of untreated controls up to the 18th hour after anesthesia. These definite and regular biochemical corollaries of the functional disturbance in ether anesthesia are not also found uniformly reflected in the urinary excretion of putrefactive bodies by surgical patients.

8985 P

Response of Chick Testes and Ovaries to Rat Pituitary Implants.*

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Implants of rat pituitaries were made into 98 male and 40 female chicks as part of a program of study of the pituitary-gonad inter-relationship. The results were unexpected, and we believe of general interest because of possible bearing on the problem of species specificity of hormone action. The bird affords an excellent test for experiments involving pituitary hormones of animals in different classes because: (1) of the marked response of the immature testis to the "follicle stimulating" hormone (F.S.H.),[1] and, (2) since it was reported by one of us,[2] that the testes of chicks given the pituitary luteinizing principle differed histologically from those of chicks which received F.S.H.

Young adult rats weighing 180-225 gm. were used as donors and those castrated were operated upon 9 weeks previous to the time of implantation. Weights of pituitaries used corresponded closely to those tabulated for Wistar rats of similar weight.[3] Single pituitaries were implanted subcutaneously into the chicks on the 5th and 7th days after hatching and the birds were killed on the 9th day. Control experiments with implants into immature female rats demonstrated the same quantitative difference in ovarian weights which has been reported by other workers.[4] A summary of the results in the chick appears in Table I.

*Contribution No. 74 from the Waterman Institute, and No. 262 from the Zoology Department of Indiana University.

1 Riddle, O., and Bates, Robert W., *Endocrinology*, 1933, **17**, 689.

2 Breneman, W. R., *Anat. Rec.*, 1936, **64**, 211.

3 Donaldson, H. H., 1924, *The Rat*, Philadelphia, 256.

4 Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 371.