Case 6. Twisted pedunculated fibroid. Myomectomy.

Case 7. Ovarian cyst, clean peritoneum. Left cystectomy.

All these patients received at the end of the operation one hundred thousand Oxford units of penicillin in 20 ml normal saline solution instilled into the peritoneum. Blood was drawn half hourly and hourly up to 8 hours for serum penicillin assays in order to estimate its rate of absorption. The blood was allowed to clot and stored in the refrigerator over night. The penicillin levels were determined by the method of Rosenblatt, Alture-Werber *et al.*[1]

Results. It can be seen from Fig. 1 that the absorption of the instilled penicillin from the peritoneum into the blood stream takes place over a longer period and is more sustained than the intramuscular or the fractional intravenous routes. Peak levels are reached 30 minutes after instillation. Amounts of penicillin sufficient to combat penicillin sensitive organisms are found 3 (Cases 5, 6, 7) to 7 (Cases 1, 3) hours after administration. Case 3 with ascites and a tuberculous process attained the highest blood levels (1.5 Oxford units per ml of serum) and 0.05 Oxford units per ml were present even 7½ hours after the peritoneal deposition.

Summary. Penicillin given by the intraperitoneal route in a single dose of one hundred thousand Oxford units is detectable in the blood stream 3 to 7 hours in 5 cases, and one to 2 hours in 2 cases in sufficient amounts to neutralize penicillin sensitive invaders in abdominal surgery.

[1] Rosenblatt, Philip, Alture-Werber, Erna, Kashdan, Florence, and Loewe, Leo, *J. Bact.*, 1944, **48**, 599.

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Chronic Oral Toxicity of Choline Chloride in Rats.*

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The demonstration of the ability of the rat to metabolize relatively large single doses of choline chloride[1] led to an attempt to discover what limits of tolerance exist for chronic administration. Two series of experiments were therefore carried out, (a) in which choline chloride at various levels was incorporated in the solid diet and (b) in which solutions of choline chloride were substituted for the drinking water.

Choline Chloride in Food. Choline chloride at levels of 0.01, 1, 2, 7, 5, and 10% of the diet was fed for periods of 3 to 4 months to groups of 5 rats each. The average body weight curves (Fig. 1) demonstrated that choline at 0.01 and 1% of the diet produced no effect on growth as compared to litter mate controls. 2.7% of choline reduced growth by about 20%, 5% of choline by about 45%, and 10% of choline in the diet permitted no growth.

Food intakes were measured daily for each group over an 8-week period (Table I., A). The lower growth rates in the groups on higher choline intakes were not a result of any specific toxic effect but were evidence of a refusal of food.

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[1] Hodge, H. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 26.

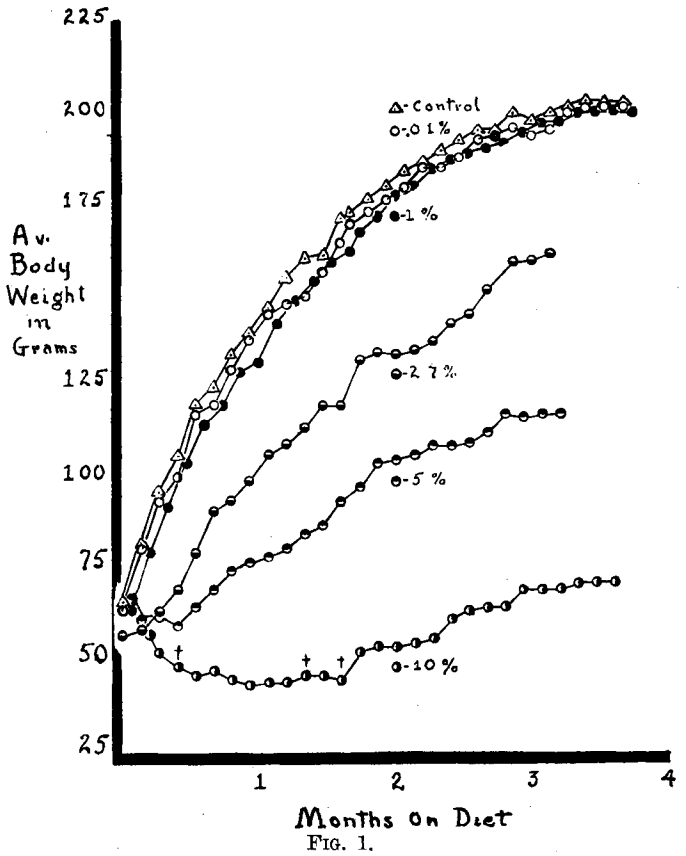

FIG. 1.

Growth curves of groups of rats on various dietary levels of choline chloride. Little effect on growth was observed in diets of 0.01 and 1.0%; progressively greater effects were obtained by including 2.7, 5, and 10% of the drug in the diets. Each + sign marks the death of one rat.

At the end of the experimental period the rats were autopsied. The organs from each rat were weighed and samples of a number of tissues were taken for histological examination. Although the smaller rats (those on the higher choline levels) had smaller organs, the average organ weights per gram rat were comparable for all groups except for the rats whose diet contained 10% choline. In these rats on the highest dosage, the organs (except the spleen) were all much *larger* per gram rat than is normal. In the rats on high choline intake almost no fat was seen either in the subcutaneous tissues, the mesentery, or the perirenal sites. The absence of fat was probably a result of the low food intake; however, the lipotropic action of choline[2] may also have been partly responsible.

The results of the histological examination conducted by Drs. J. R. Carter and H. Kattus of the Department of Pathology were largely negative. In addition to sections of the brain, stomach, kidney, heart, lung, spleen and liver, sections were also prepared of small intestine, large intestine, and ovary or testis. In the group receiving 0.01% of choline, the tissues were essentially normal. In the group receiving 1% of choline, there was a tendency toward an increase in the spleen of hemosiderin-containing phagocytes; this pigment was separately identified by special iron stains. In this group, one rat kidney showed prominent areas of lymphoid infiltration. In the liver of one rat of the group receiving 2.7% of choline, was found an area of focal degeneration. In the other groups including the con-

[2] Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1932, **75**, 405.

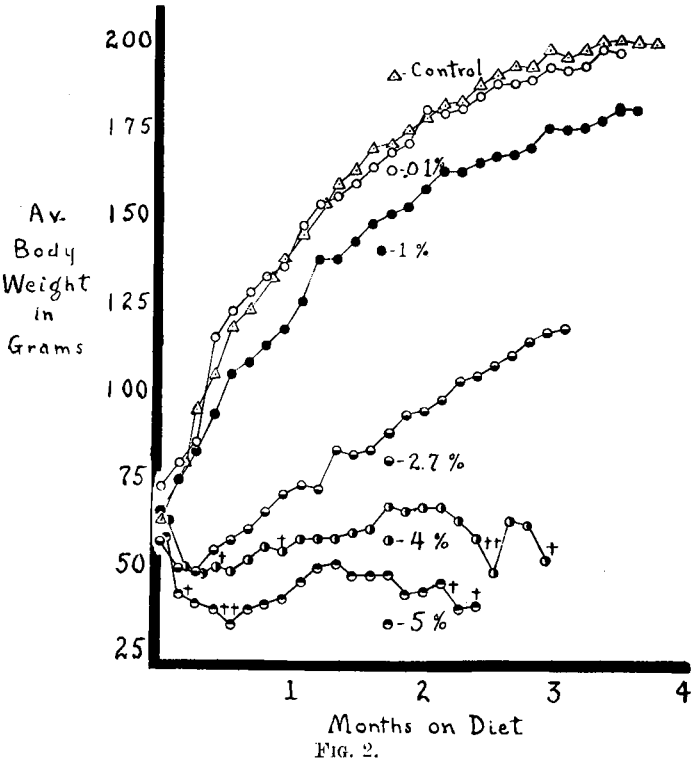

FIG. 2.

Growth curves of groups of rats drinking various solutions of choline chloride. A concentration of 0.01% produced little effect; however, increasing decrements in growth followed drinking 1.0 and 2.7% solutions. Rats given a 5% solution did not regain their original body weight during a period of over 2 months.

trol group, all sections were negative, although (particularly from rats on the higher choline intakes) the sections of many organs appeared smaller than the control tissues. In general, no consistent histopathological changes could be attributed to the inclusion of choline chloride in the food.

Choline Chloride in the Drinking Water. Choline chloride solutions containing 0.01, 1.0, 2.7, 4 and 5% by weight were substituted for the drinking water for the groups of 5 rats each whose growth curves are shown in Fig. 2. The lowest level as was found with the previous dietary experiment produced no diminution of the growth rate. However, drinking 1% choline solution produced a decrease in growth; this was in distinction to the normal growth rate of rats ingesting a 1% choline diet. A 40% reduction in growth was obtained when the rats drank a 2.7% choline solution. No growth occurred when they drank a 4% solution, and on a 5% solution, the survivors after 2 months weighed only 55 and 59%, respectively, of their weight at the start of the experiment. Other groups of rats were given solutions of 0.3, 3.0, 6.7 and 10% choline, respectively. These rats responded as would be expected from the effects seen in the groups described above. No rats drinking 6.7 or 10% choline solutions survived more than about 8 weeks and 7 of 10 rats on these levels died within 10 days.

The water consumption for each group was estimated daily during the first 8 weeks of the experiment (Table I., B). The growth curves reflected the intakes.

At the end of the experimental period the rats were autopsied and the organs treated (Table II., B) as before. The most noticeable consistent change in organ weights occurred in the livers; increasing percentages of choline gave smaller livers. However, when expressed on the basis of percentage of body weight, the average organ weights from the

TABLE I.
Food and Water Consumption by Rats Receiving Various Levels of Choline Chloride.

Choline Content %	A. Choline in diet Avg daily intake Initially g	A. Choline in diet Avg daily intake After 8 weeks g	B. Choline in water Avg daily intake Initially ml	B. Choline in water Avg daily intake After 8 weeks ml
0.01	8.7	12.6	21	33
0.1			14	21
2.7	4.5	12.3	7	12
5.0	3.3	7.3	2	4.5
10	2.1	2.5		

various groups were fairly comparable, except for the group receiving the 3% choline solution. Here the tendency was for the organ weights to constitute larger fractions of the body weight.

The histopathological examinations were carried out as previously described. In general the findings were negative. In the group receiving 0.01% choline, the spleens seemed to show an increase in the hemosiderin-containing phagocytes. In this group, 3 of 5 kidney sections showed in the cortex and pyramids a few focal collections of lymphocytes, most of which appeared to have a peri-arterial distribution. With these few exceptions, for all the other groups including the control rats the tissues were essentially normal. Again it may be said that no consistent pathological findings were observed which might reasonably be attributed to the effect of choline chloride.

Summary. Chronic feeding of a diet containing up to 1% of choline chloride produced no evidence of toxicity. Except for growth rate depression, no toxic effects of higher dietary levels have been observed. Rats which drank water containing 1% of choline chloride showed perceptible diminution in growth rate. Greater concentrations than 3% choline in the drinking water were poorly tolerated. No histopathological effects could be directly attributed to the ingestion of these amounts of choline.

TABLE II.
A. Average Weights of Various Organs of Rats Receiving Diets Containing Various Levels of Choline Chloride. (5 Rats in Each Group)

Choline HCl in diet %	Avg body wt at sacrifice	Average organ weights in grams: Brain	Stomach	Kidney	Heart	Lung	Spleen	Liver
0.01	202	1.82	0.98	1.55	0.79	1.35	0.89	6.73
1.0	200	1.64	1.03	1.58	0.70	1.03	0.51	6.58
2.7	162	1.58	1.08	1.18	0.50	0.93	0.64	5.51
5.0	118	1.59	0.90	0.99	0.46	0.63	0.44	3.86
10.0	72	1.38	0.77	0.96	0.43	0.53	0.28	3.12
Control	202	1.59	1.11	1.68	0.62	1.11	0.59	6.54

B. Average Weights of Various Organs of Rats Receiving Various Concentrations of Choline Chloride in the Drinking Water. (5 Rats in Each Group)

Choline HCl in diet %	Avg body wt at sacrifice	Average organ weights in grams: Brain	Stomach	Kidney	Heart	Lung	Spleen	Liver
0.01	199	1.65	1.05	1.60	0.67	2.02	0.64	6.27
0.3	165	1.51	0.96	1.55	0.63	0.98	0.50	5.72
1.0	183	1.70	0.90	0.90	0.70	1.10	0.70	5.10
2.7	121	1.50	0.80	1.30	0.50	0.70	0.40	4.10
3.0	76	1.46	0.70	0.98	0.36	0.67	0.25	3.46
Control	188	1.55	0.99	1.52	0.55	1.00	0.70	8.69