This chicken was bled 36 days after the inoculation. There are indications that the sera of tumor-bearing fowls in the terminal stages of the disease are not protective to the same degree, but this point is now under investigation. Here, too, as in the chicken filtrate, the protective substance seems to be associated with the globulin fraction. The failure of Andrewes and Mottram to find neutralizing substances in the sera of normal chickens is probably due to the small doses used. In our experience, rarely was a serum active in amounts of less than 2 cc. to 4 cc.

It will probably be found that this protective mechanism is not specific in its action, and that it is equally effective against the filtrates of other filterable chicken tumors. The development of metastases probably depends, as is customarily believed, upon the migration of live cells, for the active agent liberated by the breaking down of tumor cells is probably neutralized by the protective substances in the plasma.

5400

The Gastric Hunger Mechanism. II. The Effect of Diet.

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Balloon tracings of the insulin gastric hypermotility cannot be distinguished by inspection either from the type III hunger contractions, as classified by Carlson,[1] or from the post-feeding tracings described by Mulinos.[2] They can be readily identified, as follows: The post-feeding tracings are uninfluenced by food, or by the intravenous injections of glucose; the hunger type III is depressed by food, but not by glucose intravenously; the post-insulin hypoglucemic hyperactivity is abolished by raising the blood sugar, and removing the hypoglucemia. They are all abolished by adequate doses of atropine.[2]

Two series of 4 female dogs, with gastric fistulae, were studied for 6 to 18 months. The dogs were trained to lie quietly, while the gastric contractions were being recorded by the balloon method described elsewhere,[3] using a chloroform manometer. The insulin

[1] Carlson, A. J., *Am. J. Physiol.*, 1912, **31**, 151.

[2] Mulinos, M. G., *Am. J. Physiol.*, 1927, **83**, 115.

[3] Mulinos, M. G., *Am. J. Physiol.*, 1926, **77**, 158.

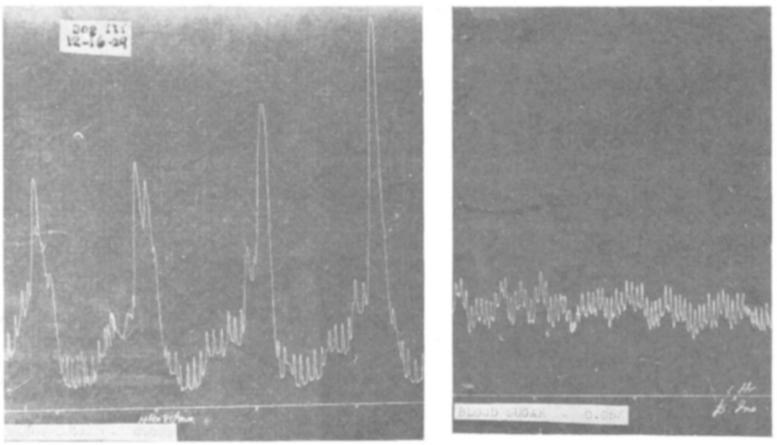

FIG. 1.
Dog balloon record of gastric motility. Gastric response to insulin hypoglucemia after 10 months on the deficient diet. (a) Normal type I contractions. (b) 1 hour after insulin, 2 units per kilo.

was injected subcutaneously or intravenously. No tracings were made within 12 hours of a feeding; the usual time was 18 to 20 hours.

In the first series, insulin hypoglucemia failed to evoke its characteristic gastric hyperactivity. Instead, it produced only an ill-defined undulatory variation in tonus (Fig. 1). The animals of this series were on a diet that consisted of one part of boiled, chopped beef and 2 parts of bread. Although they ate the whole of the portion offered each day, the dogs failed to gain in weight, and occasionally lost a little. It seemed probable that the diet was incomplete, and various modifications were tried. *The gastric response to insulin hypoglucemia returned when there was added to the diet canned vegetable soup.*

The second series of 4 animals had been kept on the above meat-bread-vegetable soup diet for several weeks. These animals gave the usual gastric hypermotility to insulin hypoglucemia. The dogs were then put on the diet consisting of bread and lean meat exclusively, as a control to the first series. Within 1 to 3 months of the change in diet, the gastric hypermotility response to insulin hypoglucemia gradually weakened, and eventually was lost. (Fig. 1.) Along with the decreased gastric response, it was found that the amount of insulin necessary to shock had to be increased by several fold (see note under Fig. 2).

Neither the source of the insulin, nor the oestral status of the animal, nor the salt content of the diet influenced the gastric re-

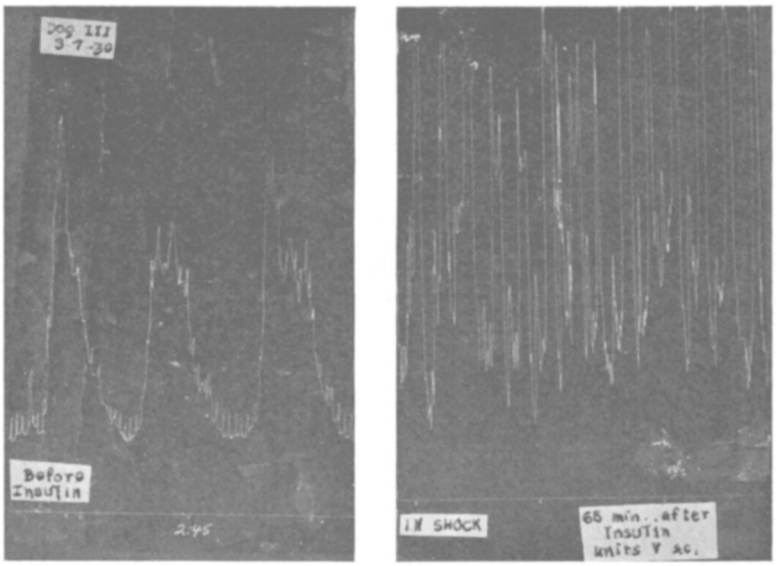

FIG. 2.
Same dog 3 months later, 6 weeks after the addition of a cake of yeast to the daily diet. (a) Normal type I contractions. (b) 1 hour after insulin, units 0.5 per kilo. Compare with Fig. 1 (b). Note the difference in the dose of insulin administered in each case.

sponse to insulin hypoglucemia. However, a week to 10 days after a cake of yeast was added to the daily diet, the stomach began to respond to insulin hypoglucemia by an increase in tonus and activity (Fig. 2).

The insulin necessary to produce shock became progressively less, until the dose was reached to which the dogs had been sensitive before the institution of the special diet. For most of the dogs, the tolerance increased from about 1 unit, on a complete diet, to 2 or 2.5 units per kilo on a bread-meat diet. It was also noted that as the dose of insulin necessary to produce shock increased, the preshock symptoms of salivation, restlessness, twitching and weakness were seldom followed by convulsions; after the addition of the yeast, small doses of insulin frequently led to unheralded convulsions, which required large doses of glucose to suppress.

Insulin, when injected intravenously in from 5 to 20 units (0.25 to 1.0 cc. of U xx) causes a transient but complete inhibition of the gastric hunger contractions, and lowers the gastric tonus. The depression lasts from 5 to 30 minutes, and is often followed by gastric hyperactivity of the hypoglucemic type. The insulins tried were those of Lilly and Mulford. A 0.1% phenol solution (insulin preservative) injected did not have the same effect. This phe-

nomenon was first observed by Simici, Guirea and Dimitriu[4] for man. Templeton and Quigley,[5] however, found that intravenous injections of insulin do not affect the main part of the stomach, but do depress a Heidenhain pouch of that organ.

Conclusions. (1) The response of the stomach to insulin hypoglucemia by hypermotility is dependent upon the presence in the diet of a sufficient amount of a yeast or vegetable factor, probably vitamin B. (2) A diet lacking this factor increases the resistance of the animal to the hypoglucemic effects of insulin, and to the convulsive effects of hypoglucemia. (3) The observation of Simici, Guirea and Dimitriu,[4] that insulin injected intravenously depresses the stomach of man, is confirmed for the dog.

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Further Note on the Enumeration of Blood Platelets and Red Blood Cells.

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In a previous communication,[1] a new method of blood platelet and red cell enumeration was proposed in which Ringer-Heparin solution was substituted for Hayem's solution as the diluent. In order to test the preliminary contention that the red blood cells may be counted in the Ringer-Heparin solution as accurately as with Hayem's solution, 75 parallel counts have since been made within 6 hours after the blood was taken. These included 24 counts upon 8 normal and pathological rabbits, 6 counts upon 1 normal and 2 anemic dogs, and 45 counts upon 31 normal and diseased humans, no material being omitted. The means of these parallel determinations are as follows: (1) Hayem's solution: 5,109,700 red blood cells per cmm. (2) Ringer-Heparin solution: 5,116,200 red blood cells per cmm. The standard error of the mean for the 75 counts was approximately the same in each case, that is, 175,000 cells. It may be concluded, therefore, that both methods gave identical results. Hemolysis or fading of the red blood cells with the use of

[4] Simici, Guirea and Dimitriu, *Arch. Malad. Appar. Digest et Malad. Nutrition,* 1927, **17**, 17.

[5] Templeton and Quigley, *Am. J. Physiol.*, 1930, **91**, 467.

[1] Casey, A. E., and Helmer, O. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 655.