in the rats receiving the drugs appeared to be empty after paraffin embedding, but were shown to be filled with fat in frozen section preparations. The small number of erythrocytes in the blood plasma in the rats and the filling of the lumens with fat may be a reason why the dilated capillaries appeared empty after paraffin embedding.

Summary. Lipemia and a glomerular lesion consisting of many fat filled spaces in the glomerular tuft appeared in from 2-4.5 months in Buffalo-strain rats fed diets containing N-N′-diacetylbenzidine and 4,4-4′, 4′-tetramethylbenzidine. No similar lesions were found in two strains of mice ingesting diets containing these two chemicals.

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Effect of Changes in Environmental Temperature on Stomach Emptying in Rats.* (22182)

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Rats transferred from room temperature to cold environment lose weight during the first few days. One obvious reason seems to be that, in spite of the increased caloric requirements in cold, the animals do not consume more food during this time than they did at room temperature. In search for the cause of this unsatisfactory food intake in experiments reported below we investigated specifically the question whether gastric emptying time is influenced by changes of environmental temperature. Although it has been shown earlier (1) that stimuli for food intake do not originate from the gastro-intestinal tract, everyday experience indicates that disturbances in physiological functions of the digestive organs may interfere with voluntary food intake. An early and important phase of food utilization is emptying of the stomach. We, therefore, considered first the changes in stomach emptying of animals exposed to lower temperature. The effect of changes in environmental temperature on stomach emptying has been discussed earlier(2) but the conclusions were equivocal. We decided, therefore, to investigate this problem directly applying a method for determination of stomach emptying time which proved to be valuable in our earlier investigations(3). The method is based on determination of N found remaining in the stomach 2 hours after voluntary consumption of a measured quantity of protein.

Methods. Male, Sprague-Dawley rats of 110-220 g weight were used. Stomach emptying time was determined by a method based on the method of Cori(4). However, we found, in preliminary experiments, that stomach emptying time varied widely after forced feeding by stomach tube. Excitement of the animals may be responsible for these variations. Consequently, adult male rats were trained to eat, spontaneously, definite quantities of protein after 24 hours starvation. Animals were maintained at room temperature of 22.2°-0.5°C. After 24 hour fast, with free access to water, each animal was presented with 2,-2,2 g of low fat tuna meat.† The N content of each batch of tuna meat was determined and the amount offered was calculated so that approximately 86 or 100 mg was consumed by the animal. Only those animals which consumed all of the tuna without spillage, within 15 minutes, were used for the experiments. After a digestion period of 2

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† "Chicken of the Sea" brand Dietetic Tuna was used after draining of the cans.

hours, with free access to water, they were sacrificed by a blow on the head. The abdomen was opened immediately and ligatures were placed at the cardio-esophageal, gastro-duodenal and ileo-cecal junctions. Stomach and small intestines with their contents intact were then removed, and after opening the lumen the contents were rinsed with distilled water into separate beakers. These washings were transferred to 100 ml volumetric flasks containing 10 ml concentrated sulfuric acid. The flasks were then placed in a boiling water bath for 90 minutes to completely disintegrate all proteinaceous material. After this digestion, samples were diluted with water to 100 ml volume. The N was determined on an aliquot part after further digestion with sulfuric acid, either by nesslerization using a Lumetron photocolorimeter, or by semi-micro Kjeldahl method. Both methods gave closely comparable results. The same procedure was followed with animals subjected to cold and heat with the following alterations: *For acute cold studies* immediately after all the tuna was eaten at room temperature, the cage with the animals was placed in a large walk-in refrigerator at 3°C for the 2-hour digestion period. *For acute heat studies,* immediately after all tuna was eaten at room temperature, the cage with the animals was placed in a dry heat room, temperature 37.8°C for the 2-hour absorption period. *For studies with cold adaptation* 5 days prior to start of experiment, animals were placed in the refrigerator at 3°C. They received food (Purina Chow) and water *ad lib.* for 5 days. The sixth day each animal was fasted 16 hours. This shorter fasting time was chosen to compensate for the increased caloric demand in the cold, and because we had found previously, that stomach and intestines were entirely emptied after 16 hours of fasting in the cold. After the 16-hour fast each animal was presented with tuna and remained at 3°C for 2 hours after consumption of food; they were then removed and sacrificed instantly. *Heat adaptation studies,* 5 days prior to start of experiment, animals were placed in a dry heat room with temperature 37.8°C. They received food (Purina Chow) and water *ad lib.* for 5 days. On sixth day animals, after 24-hour fast, were presented with tuna meat. They remained at 37.8°C for 2 hours after consumption of food and thereafter were removed and sacrificed instantly. In 3 animals stomach emptying was investigated after only one-day heat adaptation.

Results of our experiments are condensed in Table I. These data show that at normal room temperature, about 87% of the consumed N leaves the stomach within 2 hours. These values agree very well with our earlier findings. In animals exposed to *cold* after consumption of food, the amount of food left in the stomach after 2 hours was much larger. In the first 4 rats only about 63% and in the 8 rats belonging to another batch of animals, only 27% of the protein had left the stomach. We cannot account for the difference in the reaction of the 2 groups of cold exposed animals, but inspection of the data and statistical evaluation show that emptying of stomach was significantly delayed on acute exposure to cold in both groups. Consequently, we repeated the experiment with animals which were adapted to cold. The results in these rats showed that stomach emptying in animals adapted to cold was identical with that observed in normal rats at room temperature.

In the next group of experiments, animals were transferred to the hot room after consumption of food. Stomach emptying was even more delayed than after exposure to cold. In some cases the N content of stomach after 2 hours was actually higher than the amount consumed. This might indicate that a profuse gastric secretion was induced by heat, and the N content of this secretion was responsible for the increased amount of N found in the stomach(5). Further experiments on animals which had been acclimatized to heat for 5 days show that similar to adaptation to cold, adaptation to heat restores the stomach emptying to normal values. We do not know how fast adaptation occurs but the few experiments performed to date (Nos. 52-54) show that a 24-hour exposure to heat is not sufficient to restore stomach emptying time to a normal value.

To determine whether gastric digestion has

TABLE I. Influence of Environmental Temperature on Elimination of Dietary Protein from Rat Stomach.

Exp. condition	mg N consumed	2 hr after food consumption: mg N in stomach	mg N in intestines	Avg % of N emptied from stomach
At room temp.	86	6.7	14.5	
	"	6.4	14.7	
	"	5.3	14.2	
	"	10.3	14.4	91.8 ± 2.75
	100	8.7		
	"	16.3		
	"	18.6		
	"	14.		85.8 ± 3.86
Acute cold exposure	86	25.7	11.6	
	"	29.6	12.3	
	"	38.9	12.4	
	"	33.9	7.5	
	"	69.0		
	"	61.5		
	"	60.5		
	"	60.0		
	"	62.5		63. ± 6.48
	100	74.		
	"	83.		
	"	65.		
	"	69.		27. ± 4.12
After 5 days cold adaptation	86	15.9	14.6	
	"	14.0	18.2	
	"	5.0	15.3	
	"	10.1	12.2	
	100	21.3		
	"	7.3		
	"	24.5		
	"	15.0		
	"	26.7		84.9 ± 8.10
At room temp. after 5 day cold adaptation	86	12.6	12.4	
	"	14.6	18.6	
Acute heat exposure	86	55.0	13.0	
	"	57.0	16.8	
	"	68.0	17.0	
	"	54.0	12.2	
	"	67.0	11.4	30. ± 7.84
	100	100.0		
	"	116.0		
	"	131.5		
	"	132.5		
	"	133.5		0.
5 day heat adaptation	100	14.3	17.8	
	"	18.8	17.6	
	"	21.9	16.4	
	"	26.2	15.8	
	"	15.4		
	"	22.2		
	"	12.0		
	"	8.5		
	"	15.4		83. ± 5.68
After 24 hr heat adaptation	86	48.0	12.2	
	"	54.5	8.4	
	"	64.5	14.6	

been disturbed by acute changes of environmental temperature, we investigated the N present in intestinal contents 2 hours after food consumption. These experiments were based on earlier investigations in which we found that hard to digest proteins such as zein, raw eggwhite or certain soy protein preparations are emptied from the stomach at the same rate as easily digestible proteins. The intestines contained, however, much larger quantities of proteinaceous material 2 hours after feeding of zein, raw eggwhite or soy protein, than after consumption of casein or fish protein(3).

Table I shows that intestinal N content in cold and heat exposed animals was very similar to the value observed in rats at room temperature. These results seem to suggest that changes in gastric motility and not disturbed digestion may be primarily responsible for the delay in stomach emptying after acute temperature changes.

Discussion. The experiments reported above show that following a protein meal, sudden transfer of rats from room to either high or low environmental temperatures, prolongs the gastric emptying time. The question whether decreased motility, closure of the pylorus or disturbance in gastric digestion or a combination of these factors is responsible for this effect can not be definitely answered by our experiments. It seems, however, that changes of motility are the main cause. This conclusion is based on extensions of earlier experiments in which gastrointestinal propulsion of hard to digest proteins, such as zein, or raw eggwhite was studied. In those experiments stomach emptying was normal, but the intestines contained abnormally large quantities of undigested nitrogenous material (4). The finding that distribution of N between stomach and intestines of cold or heat exposed animals, is the same as that observed in control experiments, seems to indicate that disturbances of gastric digestion may not be the primary cause of the observed heat and cold effects. Our experiments indicate that after adaptation to changed environmental temperature, stomach emptying time is again similar to that observed at room temperature. We must, therefore, conclude that neither cold nor hot environmental temperature as such, but rather the sudden change of environmental temperature is responsible for the delayed stomach emptying. The common factor involved seems to be an increased epinephrine production which delays gastric emptying time. Epinephrine is produced, we found earlier, in abnormally large quantities during sudden cooling as well as sudden exposure to heat, but is not present in increased amounts after adaptation(6,7).

Summary. 1. Transfer of rats from room (22°C) to either low (3°C) or high (37°C) environmental temperature immediately following a protein meal delays emptying of stomach. 2. After adaptation to changed surrounding temperature stomach emptying was found to be again similar to that observed at room temperature.

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