maintained at greater than 120 U.S.P. units/mg and specific radioactivity of 10^5 or more cpm/mg can be obtained. The semi-synthetic process is simple enough to permit its use to further knowledge about the metabolism and mode of action of heparin.

Summary. A method for synthesis of radioactive heparin of high unit activity and radioactivity has been described using a process of N-desulfation and N-resulfation. Comparison of the biological identity of the radioactive material with commercial heparin has been made. N-resulfated S^{35} heparin appears to be similar to biosynthetic S^{35} heparin in both its clearing and anticoagulant activity as well as its ultimate fate and distribution in the body.

$ClS^{35}O_3H$ was prepared by the reaction of $H_2S_{35}O_4$ and PCl_5, followed by distillation under nitrogen. We wish to thank Marshall Draper for working out this method and supplying us with the $ClS_{35}O_3H$. The authors also wish to acknowledge the technical assistance of C. deFusco, T. Hooper and M. Horne.

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Iodoacetamide Induced Gastric Ulcers in Rats.* (27373)

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Chemicals such as acetylsalicylic acid(1), cortisone(2), histamine(3), cinchophen(4,5), and reserpine(6) when administered in proper concentration are capable of producing ulcers in stomachs of animals. Many of the experimental ulcers, however, have a tendency to perforate, heal quickly or cause profound emaciation of the animal(7). Production of gastric ulcers in a majority of animals without perforation or emaciation would therefore be advantageous in experimental studies of pathogenesis. Iodoacetamide feeding will frequently produce chronic deep ulcers in the secretory part of the rat stomach. Because of the apparent good health and sustained weight gain in these rats, it is believed that the production of ulcers by iodoacetamide feeding may provide a useful method in future investigations of pathogenesis.

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Method. Four to 6 Sprague-Dawley rats of different age and sex were housed in one open-bottom mesh cage. Control and test rats were fed Purina Chow and water *ad libitum*. One gram of iodoacetamide was dissolved in 20-30 ml of water buffered with 2% NaOH to pH 6.5-7.5 and then diluted to 100 ml. Each day either 5 or 10 ml of 1% solution was diluted further to 100 ml. Final concentration of iodoacetamide in drinking water was either 50 or 100 mg per 100 ml. Water consumption usually increased from 4 to 10 ml per day as the rats gained weight. Depending upon the variables indicated, the consumption of iodoacetamide by individual rats varied from 2.0 to 10 mg per day. A 1% solution of iodoacetic acid was prepared in a similar manner and administered in drinking water in another assay. Rats in the control and test groups were fed for periods of 20 to 93 days (Table I). When the experiments were terminated autopsies were per-

TABLE I. Observations Made in Control and Test Rats Following Iodoacetamide and Iodoacetic Acid Feeding.

No. of rats and sex		Conc. of drug in water, mg/100 ml	Period fed in days	Avg initial wt, g	Avg wt at termination, g	Microscopic alterations in stomach	
						Gastritis	Deep ulcers
11	♀	Control	49	40	172	0	0
5	♂	Iodoacetamide 100	20	125	129	5	2
12	♀	Control	42	42	185	0	0
2	♀	Iodoacetamide 100	45	150	178	2	1
4	♂	Iodoacetamide 100	90	49	264	4	3
5	♀	Control	61	43	183	0	0
6	♀	Iodoacetamide 50	93	39	205	6	3
6	♀	Iodoacetic acid 50	61	56	161	0	0

formed and the parenchymatous organs examined. Representative samples of tissue were taken from stomach, intestines, colon, liver, kidney, urinary bladder, adrenal, spleen, pancreas, lymph nodes, gonads, heart, lung, aorta, knee joint, and bone marrow for microscopic examination. The tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and routinely stained with hematoxylin and eosin.

Results. The number of control and test animals fed, period of feeding, concentration of drug administered, weight gain and alterations observed in the stomach are shown in Table I.

Iodoacetamide feeding always produced gastritis which was further complicated by chronic ulceration in many of the test rats. The ulcerations invariably developed in the secretory portion adjacent to the transverse gastric ridge near the esophageal opening (Fig. 1). Superficial ulcerations were infrequently encountered in the forestomach. None of the test rats developed duodenal ulcers. Gastric perforation did not occur in rats which were fed iodoacetamide for 93 days. Concentrations of 50 or 100 mg of iodoacetamide per 100 ml of drinking water were also compatible with weight gain and growth. Feeding 50 mg of iodoacetic acid per 100 ml of drinking water did not produce gastric ulceration. This level of iodoacetic acid was found to be toxic because the test rats did not grow as well as the controls.

Microscopic examination of tissues from control and test rats was normal except for gastritis and gastric ulceration in the iodoacetamide fed rats. Gastritis was characterized by the presence of lymphocytes and monocytes in the mucosa and submucosa. Superficial mucosal necrosis or ulceration was observed in the secretory stomach in 6 of 17 test rats. Deep gastric ulceration with destruction either of the muscularis mucosa or the muscle layers was seen in 9 of 17 stomachs from iodoacetamide fed rats. In the chronic ulcers one usually observed a necrotic base, a zone of leukocytic infiltration and an area of fibrous repair with numerous capillaries (Fig. 2). Peculiarly there was minimal or no evidence of inflammation in either the forestomach or duodenum. In 3 of 17 test rats there was an appreciable cystic dilatation of the lymphoid sinuses and hyperplasia of lymphoid follicles in the mesenteric lymph nodes. The cause for this alteration was not apparent but was presumably related to the development of chronic gastric ulcers in these test rats. Microscopic examination of stomachs from iodoacetic acid fed rats revealed no evidence of inflammation or ulceration.

Discussion. Dietary deficiency can cause gastritis and superficial mucosal ulceration in a large number of rats(8). In this study the diet was found to be satisfactory because gastric mucosal necrosis was not observed in the control rats. Administration of cortisone(2), histamine(3) or cinchophen(4,5) will produce gastric ulcers which may perforate. In the present study iodoacetamide feeding in drinking water produced chronic gastric ulceration without perforation. Production of chronic gastric ulcers in rats is intimately re-

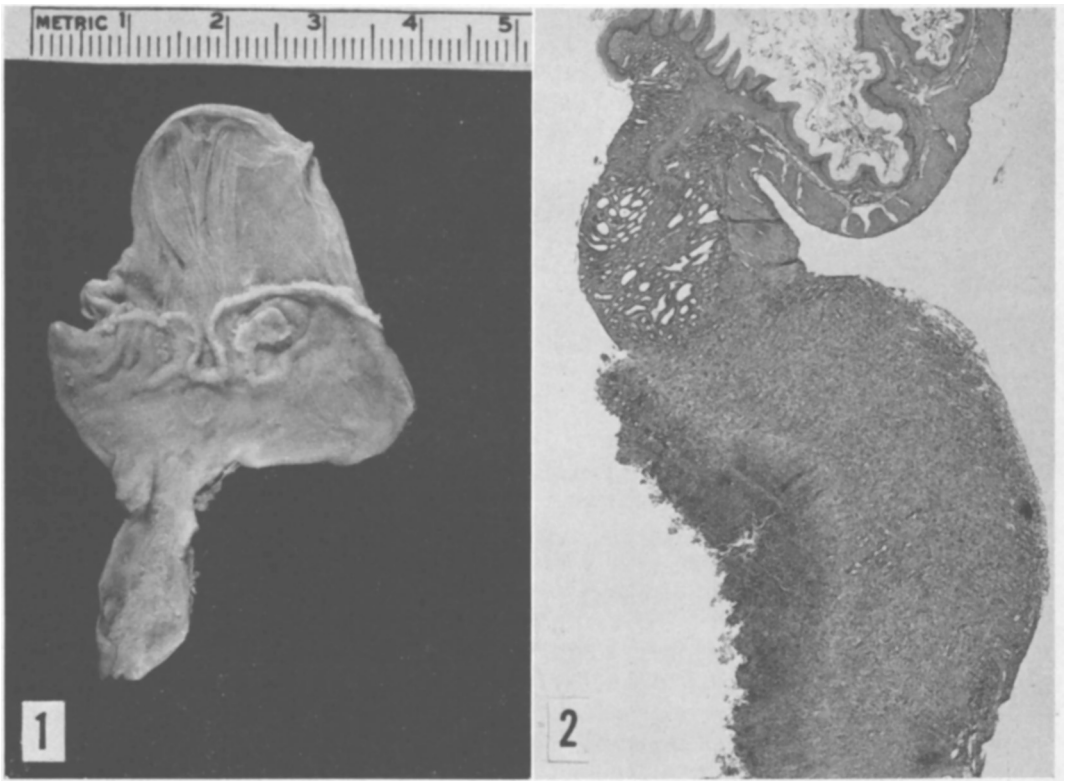

FIG. 1. Rat fed 50 mg of iodoacetamide/100 ml drinking water for 93 days. In this period the rat gained from 38 to 188 g. Note well-defined irregular ulcer distant to the transverse ridge. Mucosa in the forestomach and duodenum is normal.

FIG. 2. Rat fed 100 mg of iodoacetamide/100 ml drinking water for 90 days. Rat gained from 59 to 229 g. A necrotic base, with destruction of the muscle layers and organization are apparent in the secretory stomach (H & E, 20×).

lated to the chemical structure of the drug. Iodoacetic acid, when fed for comparable periods, did not produce gastric ulcers. Interestingly, 2 other somewhat similar compounds, acetamide(9) and thioacetamide(10, 11), produce injury of the liver without causing gastric ulceration.

The mechanism responsible for production of iodoacetamide-induced ulcers is not apparent. Iodoacetamide is frequently employed in *in vitro* studies to inactivate enzymes(12). Hollander has shown that topical application of an aqueous solution of iodoacetamide to a canine Heidenhain pouch mucosa stimulated a flow of a dilute form of mucus(13). Mucosal iodoacetamide application prior to subcutaneous injection of histamine also promoted a transient inhibition of hydrochloric acid secretion. Aqueous iodoacetamide therefore will exert an irritating effect upon the gastric mucosa and influence the secretion of hydrochloric acid. Whether ulceration observed in this study is due to direct injury of gastric mucosa by iodoacetamide or is associated with an interference of gastric secretion is not clear. Microscopic observations suggest that the gastritis and superficial mucosal necrosis somehow predispose to subsequent destruction of the muscularis mucosa and muscle layers.

Summary. Iodoacetamide when fed in drinking water at concentrations of 50 or 100 mg per 100 ml is capable of producing chronic gastric ulcers in the secretory portion of the rat's stomach. This method may prove useful in studies of pathogenesis because the gastric ulcers did not perforate or cause a serious nutritional disturbance.

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Effect of Ethanol Oxidation on Levels of Pyridine Nucleotides in Liver and Yeast.* (27374)

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In the animal organism the liver is the prime site for ethanol oxidation. In the liver ethanol is oxidized partially to acetaldehyde and acetate, whereas the complete oxidation occurs mainly outside the liver. Oxidation of ethanol and acetaldehyde both require DPN as hydrogen acceptor and Forsander *et al.*(1) showed that the DPN/DPNH ratio decreased in the rat liver during ethanol oxidation. This observation was confirmed by Smith and Newman(2).

In the present investigation the levels of di- and triphosphopyridine nucleotides in the rat liver were studied during fasting and during ethanol oxidation. The results obtained from the studies on liver were compared with changes occurring in baker's yeast during oxidation of ethanol.

Methods. White laboratory rats weighing between 200 and 250 g were used. Ethanol in a dosage of 3 mg/g was administered either intraperitoneally in 10% solution or by stomach tube in a 20% solution. The animals were killed by a blow on the neck ½ to 4 hours after administration of ethanol. The liver tissue was rapidly excised and prepared for determination of nucleotides. In the experiments on fasting animals the time of fasting varied between 2 to 5 days.

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The experiments on yeast were performed with commercial baker's yeast produced by the Rajamäki Factories of the State Alcohol Monopoly, Rajamäki. The 20% yeast suspension was aerated vigorously at 30°C and after 45 min. 38.5 mg of ethanol per g of fresh yeast was added. In this suspension the pyridine nucleotides were determined before and 5 min. after addition of ethanol without separation of the yeast.

Determination of pyridine nucleotides. DPN, DPNH, TPN and TPNH were determined in the liver extracts and in yeast preparations according to the enzymatic method of Holzer *et al.*(3). The recoveries were determined by adding known amounts of the pyridine nucleotides to tissue preparations during extraction. Recoveries for DPN, DPNH, TPN and TPNH were 93%, 91%, 90% and 86% respectively. All enzymes and substrates for the determinations were obtained from the Boehringer Biochemical Co., Mannheim.

Results. Results of the animal experiments are seen in Table I. Fasting decreases the DPN/DPNH ratio slightly from 3.95 to 3.25, mainly due to a decrease of DPN. Total DPN + DPNH is also slightly decreased by fasting. During ethanol oxidation the DPN/DPNH ratio decreases to 1.44 in the fed ani-