formation of leucocytic pyrogen(16). As yet there is no evidence that salicylate stabilizes lysosomal membranes although this has been suggested(17).

At the level of salicylate used, endotoxin fevers were substantially reduced but were not eliminated because the circulating leucocytes apparently continued to release small amounts of leucocytic pyrogen. In the *in vitro* experiments, leucocytes in the presence of salicylate continued to produce pyrogen but at a diminished rate.

Our results are not in disagreement with experimental data in the literature which indicate that the antipyretic action of salicylates is mediated through the hypothalamus. Salicylates, however, do not act *on* the hypothalamus but instead act on the leucocyte to markedly decrease the amount of pyrogen it produces. Salicylates, therefore, lower fevers in the rabbit by increasing heat loss, an action apparently mediated through the hypothalamus in response to decreased levels of circulating leucocytic pyrogen. This mechanism provides an explanation for the heretofore puzzling observations that salicylates are antipyretic only in febrile animals and that salicylates do not "stimulate" a "depressed" heat regulating mechanism(18,19). Whether the above mechanism operates in primates, including man, where sweating is a complicating factor, is not known.

Summary. Sodium salicylate is antipyretic in experimental endotoxin fevers in rabbits but is not significantly antipyretic in fevers induced by leucocytic pyrogen. The *in vitro* production of pyrogen by leucocytes is significantly decreased by salicylate. The accumulated evidence suggests that in rabbits the antipyretic effect of salicylate is not central on the hypothalamus but is peripheral on the leucocyte to inhibit formation or release of its pyrogen.

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Insulin and Insulin-Like Activity in the Bile of Rabbits. (32403)

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Early work by Houssay *et al*(1) on the physiological significance of the secretion of insulin directly into the portal circulation had suggested that the insulin secreted by the pancreas might possibly be modified by the liver. This possibility was strengthened by the studies of Samaan *et al*(2) showing that pancreatic insulin is modified during the pas sage through the liver so that a form of insulin is produced which is no longer capable of

reacting with an antiserum to pancreatic insulin. We have recently found significant amounts of immunoreactive insulin (IRI) in bile following i.v. injection of exogenous insulin into rabbits(3,4). Different forms of insulin-like activity (ILA) have been described(5,6) by a number of workers using biological methods. One of the methods used for the separation of ILA has been the addition of anti-insulin serum(6-9). The ILA and suppressible and non-suppressible bile ILA (BILA) have been measured in the hepatic bile of rabbits in an attempt to elucidate whether insulin undergoes any modification while passing from the plasma into the bile. The results obtained are described here.

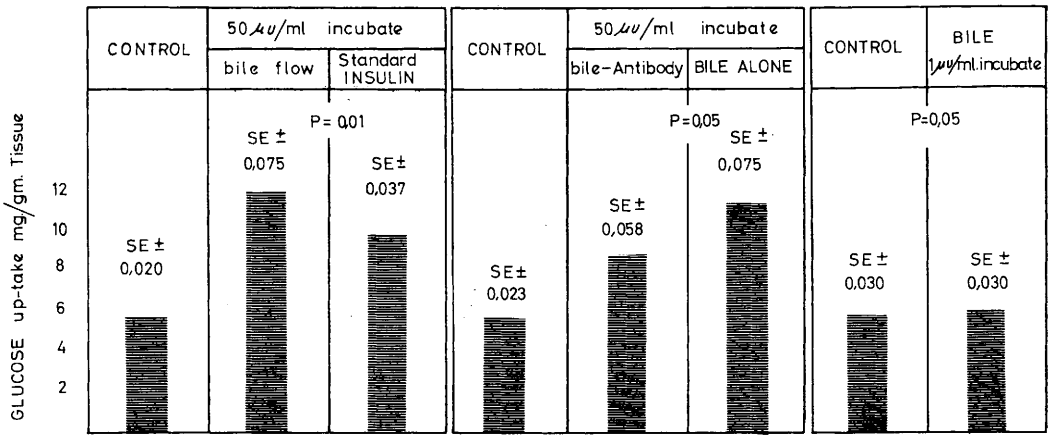
Materials and methods. Hepatic bile with a low concentration (20 μu/ml) of IRI was obtained by choledochus cannulation of rabbits. Hepatic bile with a high concentration of IRI was collected under the same experimental conditions, but after an i.v. injection of large amounts of bovine insulin in rabbits. The bile was then diluted with Krebs-Ringer bicarbonate buffer to a final insulin concentration of 1 mU/ml. IRI recovered in the bile was tested using Hales and Randle's method(10), BILA was determined following the rat epididymal fat method described below. Anti-insulin serum was added to diluted bile and the suppressible and non-suppressible BILA fractions were determined. Dry guinea pig anti-ox-insulin serum (AIS) was obtained from Wellcome Laboratories. This reagent was dissolved in diluted bile in such concentrations that 0.1 ml of the solution inhibited the effect of at least 100 μu of insulin on the rat epididymal fat pad. The bile was left at room temperature for 2 hours after addition of AIS and before the pieces of adipose tissue were added. BILA was determined with and without addition of AIS. Control experiments were done using normal guinea pig serum. The same procedure was used with standard solutions of crystalline-ox-insulin.

The details of the rat epididymal fat method were as follows: male Wistar rats (140-150 g), fed a standard diet, were killed by decapitation in groups of 10 animals. Epididymal fat bodies were removed and placed in Krebs-Ringer bicarbonate buffer, equilibrated with oxygen and CO_2. The tissues from each animal were kept in separate beakers. All tissues were handled with great care and the procedure carried out as quickly as possible. Each incubation beaker contained 2.0 ml of oxygenated Krebs-Ringer bicarbonate solution with glucose (150 mg%), crystalline serum albumin (250 mg%) and the required amount of insulin or bile (with or without AIS). The beakers were incubated in a Dubnoff metabolic shaker bath for 3 hours at 37-38°C, in an atmosphere of 95% oxygen and 5% carbon dioxide. At the end of the incubation period the tissues were removed. Glucose concentrations of the incubation fluids were determined by the Somogyi-Nelson procedure(11). Values were expressed as milligrams glucose consumed per gram of tissue.

Results. ILA of liver bile of rabbits after peripheral injection of large amounts of bovine insulin. The first panel of Fig. 1 shows the effects on glucose uptake of 1 ml of buffer containing 50 μu of crystalline insulin and of a similar quantity of IRI in aliquots of bile obtained after the peripheral injection of insulin in the rabbit. The concentration of insulin in the bile and in the standard solution was verified previously by immunologic assay. As can be seen the bile had a significant effect on glucose uptake; this was even slightly higher than that obtained with a similar concentration of crystalline insulin under the same conditions. These differences might indicate either that the bile had a greater ILA than that detected immunologically or that the bile itself has a favorable effect on the penetration of glucose into the cell by altering the permeability of the membrane directly. The ILA effect of bile samples with or without minimal amounts of insulin was studied in an attempt to clarify these possibilities.

ILA of liver bile of rabbits obtained before peripheral injection of insulin. The bile samples studied in these experiments represent normally flowing bile with an IRI concentration of 20 μu/ml. As in the previous experiment, the amount of bile added to

FIGURE 1.
EFFECT OF BILE AND OF CRYSTALLINE INSULIN ON GLUCOSE UPTAKE BY RAT EPIDIDYMAL FAT PAD
Bile insulin content was determined by the immunologic method.



ILA of a bile sample obtained after peripheral injection of insulin (30 IU).

Effect of AIS on a sample of bile obtained after peripheral injection of insulin (30 IU).

ILA of a bile sample obtained before peripheral injection of insulin.

each flask was 0.05 ml/ml of incubation mixture, representing 1 μu of insulin per ml. It is fully realized that such a small concentration of insulin is unlikely to cause an increase in glucose uptake. The third panel of Fig. 1 shows that under these experimental conditions the BILA was not significant. These results eliminate the possibility of an increase in membrane permeability in the cell induced by the bile itself.

Effect of AIS on BILA. The samples of bile studied in these experiments were obtained from rabbits after a peripheral injection of bovine insulin. The amount of AIS used was large enough to bind all the insulin present in the incubation medium. The central panel of Fig. 1 shows that the ILA of bile was not completely suppressed by incubation with an excess of specific antibody.

Discussion. The results shown above confirm the presence in the bile not only of immunologically active insulin but also of non-suppressible ILA. We have very little information regarding the nature of this non-suppressible ILA. The molecular weight of non-suppressible ILA in serum as estimated by gel filtration is 70,000-150,000(12) suggesting that suppressible insulin might be bound to another molecule. Lacy and Davies (13) and Williamson and Lacy(14) have shown that all the insulin present in the pancreas can bind to AIS. The studies of Lyngsøe *et al*(6) support the theory proposed by Samaan *et al*(7,8) that the liver is the production site of non-suppressible serum ILA. The results presented here show that not all the ILA effect in the rabbit bile obtained after the peripheral injection of insulin was neutralized by AIS, supporting the possibility that the change of suppressible insulin into the non-suppressible form might occur in the liver. These results are in good agreement with the finding of Steinke (15) that the amount of measurable ILA in rat bile was significantly higher than that of serum ILA.

Summary. Previous studies have shown that significant amounts of insulin were recovered in the bile following i.v. injections of large amounts of bovine insulin into rabbits. Suppressible and non-suppressible insulin-like activity has been measured in the hepatic bile of rabbits in an attempt to determine whether insulin undergoes any modification while passing from the plasma into the bile. The effect of bile on glucose uptake was slightly higher than that of a

similar concentration of crystalline insulin. These differences may represent the presence in bile of small amounts of ILA not detectable immunologically. Bile ILA could not be suppressed completely upon incubation with an excess of AIS. The most likely explanation for these results seems to be that insulin may undergo some modification while passing through the liver.

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Atrophy of the Lymphoid Tissues of Mice Induced by Extracts of the Submaxillary Gland. (32404)

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Extracts of the submaxillary gland of the mouse induce several unique biological effects, including the stimulation and differentiation of sympathetic nerve cells, an action on the growth and keratinization of the epidermis, the induction of granulocytosis, a renin-like action, etc.(1). Because of the presence of the last-named substance(2), we studied the effect of infarction of the submaxillary gland on the blood pressure and noted that no change in blood pressure was induced by this operation but that the thymus underwent striking atrophy. The experiments have been extended to include the effect of intraperitoneal injections of saline extracts of the gland on the lymphoid tissues and blood counts of A/Jax and C3H strain mice.

Materials and methods. The submaxillary gland of 60- to 70-day-old A/Jax mice was infarcted by ligating the artery and salivary duct of the gland with a silk thread. The animals were killed 7 days later by dislocation of the cervical vertebra, and their tissues weighed and examined histologically. Blood obtained from the tail vein was stained with May-Grünwald and Giemsa solution and 200 cells or more were counted. The data were analyzed by Student's "t" test. Extracts of the submaxillary gland were prepared by

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