observations also suggest differences between streptozotocin and alloxan action, with a considerably greater specificity of the streptozotocin effect upon B-cells than is true for alloxan. Thus, the general toxicity of the drug is very much less, particularly as far as nephrotoxicity is concerned. This greater specificity is best seen in the fact that clear-cut diabetes, although of lesser severity, has been observed with doses as low as 25 mg per kg, and diabetes of greater severity with doses as high as 100 mg per kg in the rat, while the LD_{50} in the rat has been reported to be about 130 mg per kg(3). This is in striking contrast with the action of alloxan which, in almost all species where it is effective, exhibits an extremely narrow margin between the diabetogenic and the generally toxic and lethal doses.

While much remains to be done in order to obtain a complete description of the pharmacology of the B-cytotoxic action of streptozotocin in several species, and while it is too early even to speculate as to the possible mechanism of action of the drug, it would seem clear that streptozotocin provides a new tool for production of experimental diabetes in a more graded and specific fashion than is true for alloxan. It would seem reasonable to expect that the drug may be of use in certain instances of malignant insulin-producing tumors. Finally, it is difficult not to be intrigued by the combination, in a single substance of rather low molecular weight, of 3 major biologic activities: antibiotic, anti-tumoral and B-cytotoxic.

Summary. Streptozotocin is a highly effective cytotoxic agent for pancreatic B-cells. After intravenous administration of 65 mg streptozotocin per kg, damage to B-cells is apparent as early as one hour after intravenous administration of the drug. Frank necrosis associated with phagocytosis is best seen after 7 hours, when pancreatic insulin release and hypoglycemia are also noted. By 24 hours, pancreatic insulin content is reduced to 5% of normal or less. While the B-cytotoxic effects of streptozotocin resemble those of alloxan, their specificity is very much greater, as demonstrated by the wide margin between diabetogenic dose and general toxicity.

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Studies on the Antipyretic Action of Salicylates.* (32402)

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Recent work on the pathogenesis of fever suggests that most fevers are caused by the action on the central nervous system, probably the hypothalamus, of an endogenous pyrogen released from leucocytes, as shown in Fig. 1(1-4). Similarly, defervescence of fever after treatment with salicylates has generally been attributed to their direct action on the hypothalamus, with resulting peripheral vasodilation and heat loss(5-8). Al-

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though this mechanism of salicylate action has been accepted, an equally attractive alternative occurred to us: that salicylates exert their antipyretic effect outside the central nervous system either by preventing the formation or release of leucocytic pyrogen by the white blood cells, or by destroying leucocytic pyrogen before it reached the brain. The experiments reported here were designed to determine the way in which sodium salicylate reduces fever in rabbits.

Materials and methods. As most of the experimental work on fever in the past has been done with rabbits, we used New Zealand white rabbits of both sexes weighing between 2 and 2½ kg and less than one year of age. The animals were housed and all studies were performed in air conditioned rooms maintained at 70°F. Rectal temperatures were measured with a Yellow Springs Telethermometer. Animals with initial rectal temperatures of over 39.8°C were excluded from the experiment. The fever index was calculated by measuring the area under a 2-hour fever curve as recommended by Bornstein *et al*(9). The leucocytic pyrogen (L.P.) used in these experiments was prepared from rabbit peritoneal exudate cells as previously described(9,10). Several lots were pooled, diluted if necessary to provide a uniform preparation, and stored in sealed ampules at −70°C. The bacterial endotoxin was prepared from *Salmonella abortus equi*. The minimum pyrogenic dose (M.P.D.) in the rabbit was 0.007 μg. All glassware and equipment used in these studies was made pyrogen free by heating at 180°C for 2 hours in a hot-air oven. The distilled water used for the reagents was prepared by double distillation. The second distillation was performed under pyrogen-free conditions. The distilled water was periodically tested for the presence of pyrogens in two ways: by direct intravenous injection (I.V.) of 10 ml into rabbits and by intravenous injection of 10-20 ml of a saline solution made from it then incubated with rabbit buffy coat cells. By the latter procedure we have found that as little as 1×10^{-9} g of purified endotoxin can be detected. Under these conditions there was no detectable endotoxin present in the distilled water. The sodium salicylate was rendered endotoxin free by overnight treatment with 5% sodium hydroxide and then adjusted with HCl or NaH_2PO_4 to pH 7.0 and diluted to maintain isotonicity. Sodium salicylate was given intraperitoneally (I.P.) at a dose of 250 mg per kilogram, which was found to give adequate antipyretic effect in rabbits(11,12). All cell incubations were carried out at pH 7 to 7.2. The blood levels of sodium salicylate were determined by the method of Trinder(13).

Experimental results. I. In vivo *experiments.* 1. In the first experiment with endotoxin, 19 rabbits were given sodium salicylate I.P. (250 mg/kg) and 9 rabbits remained untreated as controls. Phosphate buffered saline solution given I.P. to controls had no effect on fevers and therefore was not routinely given. One hour after the rabbits had been given salicylate, all 28 animals were given endotoxin I.V. and their rectal temperatures were recorded continuously for several hours.

As a control experiment, endotoxin (0.01 μg) was incubated for 4 hours *in vitro* with sodium salicylate buffered at pH 7.2 and then injected I.V. into 5 animals to determine whether sodium salicylate would inactivate endotoxin. The average response of the 3 groups of animals to endotoxin is expressed as fever index (F.I.) and is shown in Table I. The antipyretic effect of sodium salicylate on endotoxin-induced fever is clearly shown and, as expected, salicylate has no effect on endotoxin.

TABLE I. Effect of Sodium Salicylate on Fevers Induced by Bacterial Endotoxin.

Animal treatment	No. of rabbits	Fever index, mean ± S.E.
Intravenous endotoxin, .01 μg	9	9.0 ± 1.1
Salicylate* and intravenous endotoxin, .01 μg	19	‡3.5 ± .6
Intravenous endotoxin, .01 μg†	5	8.4 ± 1.7

* Sodium salicylate 250 mg/kg given I.P. 1 hr before I.V. injection of endotoxin.

† Preincubated with 4 mM sodium salicylate.

‡ Difference between this value and that for control animals receiving endotoxin alone is highly significant ($P < .001$ Students' "t" test).

2. In the second experiment 23 animals were treated with sodium salicylate I.P., as in the previous experiment, and 16 animals remained untreated as controls. One hour later all 39 animals received I.V. 4 ml of a solution containing leucocytic pyrogen. The resulting fever indices are shown in Table II.

TABLE II. Effect of Sodium Salicylate on Fevers Induced by Leucocytic Pyrogen.

Animal treatment	No. of rabbits	Fever index, mean ± S.E.
4 ml intravenous leucocytic pyrogen	16	6.2 ± .4
Salicylate* and 4 ml intravenous pyrogen	23	†5.0 ± .5

* Sodium salicylate 250 mg/kg given I.P. 1 hr before I.V. injection of L.P.

† Difference between this value and that for controls receiving L.P. alone is not significant (P >.1 Students' "t" test).

The average change in rectal temperature of 4 rabbits is shown in Fig. 2. This experiment shows clearly that sodium salicylate has no effect on the pyrogenic response of the rabbit to leucocytic pyrogen.

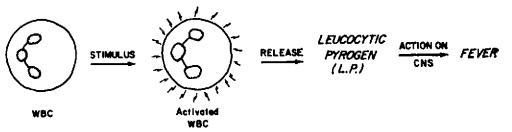

FIG. 1. A normal leucocyte (left) apparently may be activated by any number of stimlui — among them, endotoxin, viruses, phagocytosis of bacteria or inert particles. The activated leucocyte releases its pyogen (L. P.) as has been demonstrated to occur *in vitro* and *in vivo* in a number of animals including man. The leucocytic pyrogen circulating in the blood then apparently acts on the hypothalamus to cause an increase in the amount of heat retained by the animal.

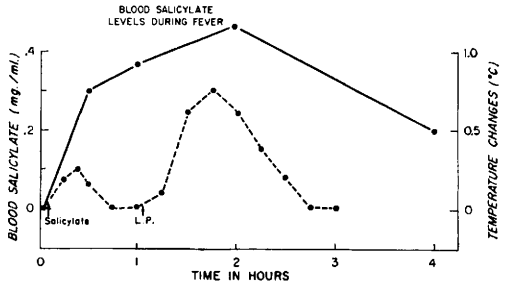

FIG. 2. Four rabbits were injected intraperitoneally with 250 mg/kg of sodium salicylate at time indicated by the arrow. Rectal temperatures (O - - - - O) and blood salicylate levels (O———O) were determined. At the point indicated, each animal received intravenously 4 ml of leucocytic pyrogen (L. P.) Salicylates do not suppress fevers induced by leucocytic pyrogen.

The average peak levels of salicylate obtained with doses of 250 mg/kg varied between 0.25 and 0.48 mg/ml. The average blood levels attained in 4 rabbits are shown in Fig. 2. There was no difference in blood salicylate levels between normal and febrile animals. These results are typical of those obtained in all experiments with salicylate treated animals.

II. In vitro experiments. 1. Peritoneal exudate cells were harvested and used to study the effect of sodium salicylate on the release of leucocytic pyrogen by these cells *in vitro*. To 10 ml of a suspension containing 1×10^8 exudate cells in phosphate buffered saline (pH 7.0-7.2) 0.2 ml of a solution of sodium salicylate, adjusted to pH 7, was added giving a 4 mM solution of sodium salicylate. The cells were incubated overnight at 37°C. Control cells were incubated in the same manner but without sodium salicylate. In 5 experiments 0.2 ml of the sodium salicylate was added to control preparations after incubation. There was no significant antipyretic effect due to this small amount of sodium salicylate. After incubation the cells were removed by centrifugation and the supernatant fluid injected I.V. into rabbits and the fever indices were calculated. Table III shows the effect of sodium salicylate on the

TABLE III. Effect of Sodium Salicylate on *in vitro* Release of Leucocytic Pyrogen from Rabbit Peritoneal Exudate Cells.

Sample treatment†	No. of samples	Fever index,* mean ± S.E.
1×10^8 exudate cells incubated in buffered isotonic saline	16	5.0 ± .7
1×10^8 exudate cells incubated with 4 mM sodium salicylate in buffered isotonic saline	50	‡3.0 ± .4

* Fever index mean is calculated from fever indices resulting from injection of an aliquot of supernatant fluid of indicated number of samples into individual rabbits.

† All cells were incubated for 18 hr in phosphate buffered isotonic saline.

‡ Difference between this value and that for controls incubated without salicylate is probably significant (P <.05 Students' "t" test).

in vitro release of leucocytic pyrogen from exudate cells. The average fever index of the animals given fluid from salicylate treated cells was nearly one-half that of rabbits given fluid from control cells. However, the range of values obtained was so great that the difference ($P<0.05$) between the two sets of data is only probably significant because exudate cells are known to contain leucocytic pyrogen within their cytoplasm when they are harvested(14). The effectiveness of this preformed leucocytic pyrogen does not appear to be decreased by the sodium salicylate.

2. Rabbit buffy coat leucocytes which contain no preformed pyrogen were used in the next series of experiments. Rabbit blood was obtained by heart puncture, centrifuged at 250 $\times$ *g* at 4°C for 20 minutes and the buffy coat removed by pipetting. The buffy coat cells were washed in physiologic saline solution twice and then resuspended in phosphate buffered saline solution. The cell suspension was divided into lots, each containing 5×10^7 cells per 10 ml. To each 10 ml of suspension, 0.003 μg of endotoxin and 0.2 ml of a pH 7 buffered sodium salicylate solution were added, giving a final sodium salicylate concentration of 4 mM in contact with the cells. To the control cell suspensions endotoxin but no sodium salicylate was added. All cell suspensions were then incubated at 37°C for 5 hours. The cells were then removed by centrifugation and the supernatant fluid was injected I.V. into test rabbits. The average value of the fever index obtained for control cells and for salicylate treated cells is shown in Table IV. There is a significant decrease in leucocytic pyrogen released from the cells treated with sodium salicylate as reflected by the lowering of the fever index.

3. The above experiment was repeated except that endotoxin was added 30 minutes before the addition of sodium salicylate in order to determine whether, after endotoxin has been absorbed by the leucocytes, salicylates would inhibit the release of leucocytic pyrogen. There was no difference in the amount of inhibition whether endotoxin was added before or after the salicylate. This is shown in Table IV.

TABLE IV. Effect of Sodium Salicylate on *in vitro* Release of Leucocytic Pyrogen from Rabbit Buffy Coat Leucocytes Stimulated with Endotoxin.

Sample treatment†	No. of samples	Fever index,* mean ± S.E.
5×10^7 cells incubated with .003 μg endotoxin	11	6.8 ± .5
5×10^7 cells preincubated 30 min with 4 mM sodium salicylate, then .003 μg endotoxin was added	12	‡3.9 ± .5
5×10^7 cells preincubated 30 min with .003 μg endotoxin, then 4 mM sodium salicylate was added	5	4.1 ± .5

* Fever index mean is calculated from fever indices resulting from injection of an aliquot of supernatant fluid of indicated number of samples into individual rabbits.

† All cells were incubated for 5 hr in phosphate buffered isotonic saline.

‡ Difference between this value and that for controls incubated with endotoxin alone is significant ($P < .01$ Students' "t" test).

Discussion. Our results show that sodium salicylate inhibits endotoxin fevers but does not inhibit leucocytic pyrogen fevers, suggesting that salicylates do not act on the hypothalamus *directly* but instead act on the fever producing cycle prior to the appearance of leucocytic pyrogen in the blood. Our *in vitro* experiments (Table III) show that salicylates in fact do inhibit the formation of leucocytic pyrogen. Since the molar concentration of salicylates used in these experiments is of the same magnitude as that in the blood of salicylate treated rabbits, it seems most probable that salicylates similarly inhibit the formation of leucocytic pyrogen *in vivo*.

The antipyretic effect of salicylates is the same whether endotoxin is added to the cells 30 minutes before or 30 minutes after addition of salicylates (Table IV). Therefore, salicylates appear to inhibit the formation or release of leucocytic pyrogen by the cell, rather than prevent activation of the leucocyte by endotoxin.

Although we can only speculate on the possible mechanism of action of salicylates, a likely possibility involves stabilization of the specific granules (lysosomes). It has been suggested by Petersdorf and Shulman (15) and we have presented further evidence, that lysosomes may be implicated in the

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