cannulated (23 gauge stainless steel cannula guides) under sodium pentobarbital (35 mg/kg) anesthesia. The tip of the cannula was aimed for the anterior preoptic area of the hypothalamus[12]. 1–2 weeks after surgery, the rats were given a central injection of 2 μg of carbachol in a volume of 1 μl of isotonic saline solution. Body temperatures were recorded by means of a thermistor probe that was inserted 6–8 cm into the rectum and taped loosely to the tail. The animals were virtually unrestrained while temperatures were being recorded.

In the second experiment male Hooded-Wistar rats, approximately 260–320 g and 90 days old, were used. They were unilaterally cannulated in the anterior preoptic area at one of 2 sites: AP 1.7, ML 0.8–1.0, DV −8.5[11] or AP 1.8, ML 1.5, DV −8.5[12] in order to produce eithera hyper- or hypothermic response, respectively. Body temperatures were recorded after either 2 μg carbachol in 1 μl isotonic saline[12] or 5 μg carbachol in 0.5 μl isotonic saline[11].

The results of these experiments are summarized in the table. There was a mean decrease of 2.7 °C in 6 of the 11 female rats which exhibited any obvious change in temperature. Histological examination revealed that those 6 which exhibited hypothermia had their cannula tips in the lower boundary of the anterior preoptic area, while those which did not respond were found to have their cannula tips below the lower border of the anterior preoptic area.

Many of the male rats dislodged their cannula guides before they could be tested. Both animals which were given 2 μg of carbachol in 1 μl of isotonic saline at the coordinates used by Overstreet et al.[12] exhibited hypothermia. So did those animals which had cannulae aimed at the coordinates of Avery[11], as can be seen in the table. Hypothermia was observed regardless of the dose or volume of injections. Control injections of isotonic saline produced no obvious temperature variation.

These results have confirmed the reports of many previous workers[1–6,12] which have shown that intrahypothalamic administration of cholinergic agonists produces a hypothermic response, but have failed to replicate the findings of Avery[7–11] of hyperthermia following intrahypothalamic injections. Since the present experiments used the same anatomical coordinates, parameters of injection and strain and sex of rats used in previous conflicting reports, these variables do not appear to be the critical ones in accounting for differences in findings. However, because of loosening of the cannulae, the actual sites of injections could not be conclusively pin-pointed. Therefore, the possibility that there are 2 cholinergic thermoregulatory centres, one for heat loss and one for heat gain, must remain open.

Nevertheless, it should be emphasized that the evidence for a cholinergic system mediating heat loss mechanisms in rats in much more compelling. One example is that atropine has been shown to block the hypothermic effects of centrally administered carbachol[13], but there is no evidence on whether it blocks the hyperthermic effects of this cholinomimetic. Until such evidence is forthcoming, we suggest that the cholinergic system in the anterior preoptica area is predominantly involved in heat loss mechanisms in rats and that the models of thermoregulation previously reported[9,10] should be regarded with caution.

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Demonstration of microtubule independent protein secretion from rat liver

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Summary. The induced secretion of rat liver microsomal β-glucuronidase into serum is, unlike other proteins secreted from liver, not dependent upon an intact microtubule apparatus.

Secretion in many cell systems has now been documented to be a microtubule dependent, colchicine inhibitable, cellular process. Studies on rodent liver, both in vitro and in vivo, have indicated that the secretion of newly synthesized albumin into serum or incubation media is markedly depressed by colchicine or other agents which disrupt the microtubule network[1,2]. These studies have shown that total hepatic protein synthesis, as measured by amino acid incorporation, is unaffected by the experimental conditions imposed. A build-up of labelled albumin has been detected within hepatocytes in the presence of the anti-microtubule agents coincident with the depression of secretion. Similar findings have been reported by groups studying fibrinogen[3] and lipoprotein[4] secretion from liver into serum. Although morphologic evidence for complete microtubule disruption has not always been obtainable[1], the lack of any effect of lumicolchicine[1,3] suggests a microtubule site of action for the colchicine effect.

The administration of any one of several organo- phosphates to rats has been shown to elicit a specific rise in serum β-glucuronidase levels[5]. This rise is independent of new protein synthesis and the source of the secreted enzyme is the microsomal pool of β-glucuronidase contained in hepatocytes[6]. Microsomal markers including glucose-6-phosphatase and microsomal albumin (immunologically detectable) are unaffected by organophosphate injection. In light of the aforementioned studies, the dependence of diisopropylfluorophosphate (DFP) induced secretion of β-glucuronidase on an intact microtubule system was evaluated.

Fasted, anesthetized 200-g female Wistar rats were used for all studies. At zero time, 25 μCi of ^{14}C D-leucine was injected into the femoral vein with or without colchicine

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Effect of colchicine on DFP induced serum β-glucuronidase and ^{14}C leucine incorporation

Zero time	10 min	Serum β-glucuronidase[a]	CPM in serum[b]	CPM in liver[c]
^{14}C Leu[d]	DFP[e]	4.9 ± 0.84	9117 ± 1483	1011 ± 35
^{14}C Leu + colchicine[f]	DFP	4.8 ± 0.85	5619 ± 442	1423 ± 144
^{14}C Leu	Saline[g]	0.10 ± 0.05	8590 ± 1291	936 ± 87
^{14}C Leu + colchicine	Saline	0.07 ± 0.02	5845 ± 946	1383 ± 271

[a] μmoles phenolphthalein released/h/ml at 70 min. [b] TCA precipitable counts in 50 μl aliquots, 70 min. [c] TCA precipitable counts in 50 μl aliquots of 1 g homogenized liver diluted in 20 ml buffer. [d] 25 μCi via femoral vein. [e] 1.25 mg/kg, i.p. in 0.25 ml saline. [f] 40 μmoles/200-g-rat, via femoral vein. [g] 0.25 ml, i.p. Data are means ± SD.

(Sigma Chemical, St. Louis, MO.) (40 μmoles). At 10 min, DFP (1.25 mg/kg) or saline (0.25 ml) was injected i.p. At 70 min, the animals were sacrificed by cardiac puncture and 50 μl of serum was added to 1 ml of 5% trichloroacetic acid (TCA) and centrifuged for 1 min in a Beckman microfuge. The TCA insoluble pellets were washed twice with 5% TCA and resuspended in 1 ml 0.1 N NaOH and 9 ml Biofluor (New England Nuclear, Boston, Mass.) for counting. An additional 25 μl serum sample was used to determine serum β-glucuronidase activity[7]. Liver protein synthesis was evaluated by measuring incorporation of ^{14}C-leucine into the TCA insoluble pellet from a 50 μl aliquot of liver homogenate. The homogenate was prepared, using a polytron homogenizer from 1 g of liver in 20 ml of buffer. An aliquot of the homogenate was treated identically to the serum sample just described.

As can be seen in the table, colchicine treatment significantly impaired the appearance of TCA-precipitable counts in the serum and led to the build-up of TCA-precipitable counts in the liver. Calculations on individual animals indicate that total incorporation of ^{14}C-leucine into TCA insoluble material was not adversely affected, but that these doses of colchicine inhibited the secretion of newly synthesized protein. This is in marked contrast to the DFP induced secretion of β-glucuronidase into the serum of colchicine treated rats which was unaffected by the massive colchicine dose utilized. This indicates a clear distinction between the DFP induced secretion of β-glucuronidase and the bulk of the protein synthesized and secreted into the serum by hepatocytes[1-4]. An explanation as to why the secretion of liver microsomal β-glucuronidase is apparently unique in its microtubule independence is not readily apparent. Induced secretion of stored or slowly turning over cellular products from other cells has been shown to be inhibitable by colchicine[8], so it is unlikely that these properties alone would lead to microtubule independence. However, in the liver this has not been evaluated and, indeed, this finding could represent a fundamental distinction between intercellular packaging and secretion of proteins that are normally stored by the hepatocyte (β-glucuronidase) and those proteins that are readily secreted after synthesis. The lack of susceptibility to colchicine or vincristine (not shown) inhibition can be interpreted in one of two ways. First, it is possible that the secretion of microsomal β-glucuronidase occurs by a mechanism truly independent of microtubules. Alternatively, the secretion may be microtubule dependent, but involve a pool of microtubular structures that are not available for interaction with colchicine or vincristine. Experiments to date do not rule out either interpretation. However, the existence of an extensive pool of colchicine insensitive microtubular structures is consistent with the findings of Redman et al.[1]. The experiments presented here provide an indication that the control of protein secretion from the liver may be even more complex than originally envisioned.

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Lead potentiation of endotoxin lethality in rats: Lack of effect of kininase inhibition

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Summary. Although lead and SQ_{20881} are potent in vitro inhibitors of kininase II activity, SQ_{20881} does not alter the sensitivity of rats to endotoxin. These results indicate that marked changes in plasma kininase activity do not contribute to endotoxin morbidity and that kininase inhibition is not the mechanism whereby lead ions sensitize rats to endotoxin.

Lead ions markedly sensitize rats to small quantities of endotoxin and elucidation of the mechanism of this synergism may clarify the toxic action of bacterial lipopolysaccharides. This report examines the hypothesis that one role of lead may be to inhibit a kininase enzyme which is responsible for degrading the hypotensive peptide, bradykinin. In this regard, endotoxin treated animals do have increased levels of circulating bradykinin[3] which may be the result of impaired kinin degradation as well as endotoxin activation of Hageman Factor and subsequent kallikrein formation. Under normal conditions,

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