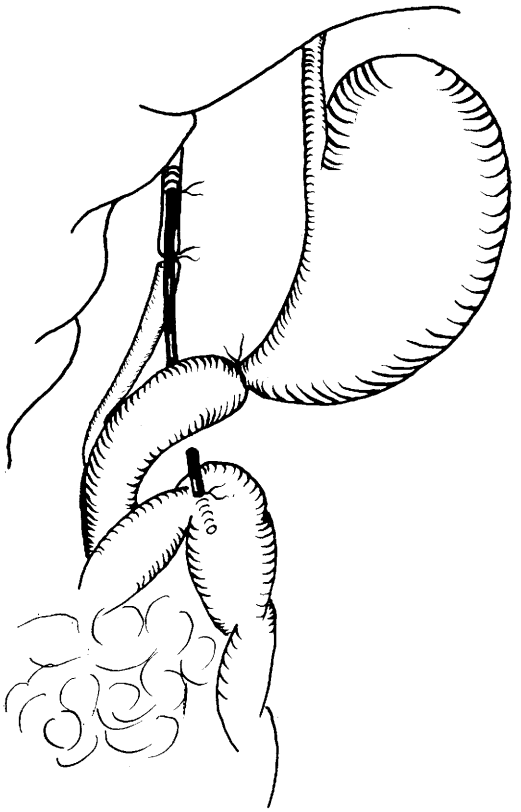

FIG. 1. Proximal portion of bile duct catheterized with fine polyethylene tube. Duct is ligated below point of insertion of tube. Free end of cannula is lead through mesentery of duodenum and implanted into rectum. With this technic entire bile output is diverted from small intestine and coecum. Pancreatic secretions reaching intestine via distal segment of bile duct are left undisturbed.

gastric secretion is related to increase in level of bile salts circulating in the pool intestine-portal blood-liver, and intraperitoneal administration should therefore be more effective. Amounts administered (23-30 mg/200 g) do not appear excessive in view of the fact that the rat excretes approximately 10-17 mg of cholic acid/hour(7) in the bile. At present, the mechanism by which parenterally administered bile salts inhibit gastric secretion in the rat cannot be explained. That this does occur is interesting since bile salts are naturally occurring compounds. Increased resorption of bile salts during digestion and absorption of food in the intestine may play a part in the physiological mechanisms of gastric inhibition following gastric emptying.

Summary. Gastric secretory activity (volume and free HCl concentration) in the pylorus ligated rat is inhibited by bile duct ligation. This is not due to removal of bile from the intestine but appears to be due to increase in level of circulating bile constituents, particularly bile salts, resulting from obstruction to the outflow of bile from the liver.

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Urinary Metabolites of Chlorpropamide in Dogs, Rabbits, and Man.* (25055)

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During pharmacological investigation of chlorpropamide [1-(*p*-chlorobenzenesulfonyl)-3-n-propylurea](1), a hypoglycemic agent, urinary excretion of the drug was measured by the method of Spingler(2). From 30 to 40% of orally administered chlorpropamide was detected in dog urine. This suggested that more than 50% of the ingested dose was metabolized. This report is concerned with identification of chlorpropamide metabolites in dog, rabbit, and human urine.

Methods. Twenty-four hour urine speci-

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mens taken after oral chlorpropamide administration were acidified to pH 2 and extracted 16 hours with methylene chloride in a continuous extractor. The methylene chloride solutions were evaporated and the residue dissolved in methanol in preparation for paper chromatography. Aliquots of the methanol solution were spotted on Whatman No. 1 filter paper and developed 16 hours at room temperature in an ascending manner with butanol saturated with 5 N ammonium hydroxide. The sheets were dried at room temperature, then immersed in freshly prepared saturated aqueous solution of mercurous nitrate according to the method of Deininger (3). After one minute of rinsing in distilled water the sheet was examined by placing it on glass plate. This permitted location of major spots by observation of the reduced translucence of the wet paper. The appearance of a gray spot as the result of reaction of sulfonamides with mercurous nitrate could also be observed. To make the spots more visible the wet sheet was slowly moved through 1 : 3 acetone dilution of saturated acetone solution of *p*-dimethylamino-benzalrhodanine. The spots then appeared reddish brown against a yellow-brown background. The sheet was then dried at room temperature. The quantity of compounds was estimated by spectrophotometry. After detection of the spots, the corresponding areas of an adjacent lane were cut out for elution. Elution was accomplished by immersing the section under investigation in .01 N sodium hydroxide solution and slowly agitating it for one hour. Ultraviolet absorption curve was checked against that of model compound of like mobility, chromatographed, and eluted in the same way. Only those runs in which the unknown absorption curve was identical with that of the known were considered in the quantitative determination. For isolation of metabolites, urine extracts were spotted on Whatman No. 1 or 3 MM paper and developed as before. Horizontal strips were cut out corresponding to the R_f of various spots as determined by mercurous nitrate-*p*-dimethylaminobenzalrhodanine treatment. These horizontal strips were eluted with methanol, the eluates were evaporated, and infrared absorption curves of the residue determined. Crystalline products were identified by x-ray powder diffraction methods.

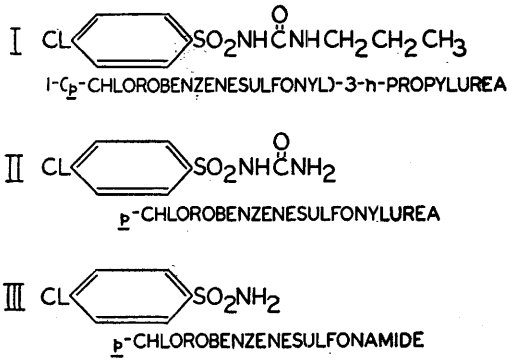

FIG. 1. Urinary excretion products of chlorpropamide.

Results. 1. *Dog.* Paper chromatograms of chloroform or methylene chloride extracts of urine from normal dogs receiving daily oral doses of chlorpropamide were compared with extracts from normal untreated dogs. Three additional spots (R_f 0.82, 0.70, and 0.42) were produced by extracts from treated dogs. The most mobile of these spots appeared gray after the mercurous nitrate reaction while the other 2 appeared as white precipitates. All 3 were clearly visible after reaction with *p*-dimethylaminobenzalrhodanine.

Chlorpropamide had an R_f of 0.71 in this system, therefore the middle spot was attributed to unchanged drug.

Two possible metabolites, (Fig. 1) *p*-chlorobenzenesulfonylurea (II) and *p*-chlorobenzenesulfonamide (III), were made available by Dr. M. V. Sigal, Jr. of these laboratories. When these compounds were chromatographed, the sulfonamide (III) gave a gray spot (R_f 0.82) with mercurous nitrate and sulfonylurea (II) had a mobility corresponding to slowest moving of the 3 spots in question (R_f 0.42).

A quantitative determination of the 3 concentrations as they occurred after chromatographic development was made by determination of ultraviolet absorption of the eluant of each area at the absorption maxima observed for each of corresponding model compounds. Twenty-four hour urine samples from a dog maintained on daily oral dose of 0.5 g of chlorpropamide contained from 27 to 33%

of administered dose as unchanged drug. The sulfonylurea (II) represented 35 to 40% of the dose, and the sulfonamide (III) an additional 16 to 24%, giving a total recovery from 78 to 97% of administered drug.

For additional identification of the 3 urinary excretion products, the infrared absorption curves of eluants from appropriate areas of paper chromatograms of urine extracts were compared with curves of model compounds and were in agreement in each case. Crystals from a benzene solution of the most mobile spot (R_f 0.82), from sublimation under vacuum at 110°C for the next most mobile spot (R_f 0.70), and from an alcohol: water solution of the least mobile spot (R_f 0.42) had x-ray powder diffraction patterns identical with the model compounds crystallized under same conditions.

2. *Rabbit.* A 24-hour urine sample from normal rabbit that had received 100 mg of chlorpropamide orally was extracted and chromatographed. One large spot appeared in addition to those attributable to normal rabbit urine constituents. This spot had the same mobility as chlorpropamide. The ultraviolet absorption spectrum of the eluant was identical with that of chlorpropamide. The 24-hour excretion was 86 mg or 86% of the dose as unchanged drug. The fate of remaining 14% has not been determined.

3. *Man.* Twenty-four hour urine samples from 3 diabetic patients who had received 0.3 to 1 g of chlorpropamide orally were extracted and chromatographed. Spots corresponding to sulfonamide (III) and the unchanged drug (I) were seen. The sulfonylurea spot, if present, was difficult to distinguish because of interfering substances present in human urine. One of the subjects received 1 g of chlorpropamide on the first, second and fifth day of treatment. Only urine specimens 24 hours subsequent to each dose were available for investigation. The extract from final of these specimens produced a spot (R_f 0.55) that has yet not been identified. The *p*-chlorobenzenesulfonamide was identified by x-ray powder diffraction pattern as in the dog. The infrared absorption curve from the supposed chlorpropamide was identical with chlorpropamide except for some additional absorption that was probably due to carbonyl contamination. A sublimation attempt yielded sulfonamide (III) instead of the expected unchanged drug. No sulfonylurea has been identified.

Discussion. Excretion of chlorpropamide and its metabolites in dog urine accounted for 78 to 97% of amount of drug administered daily. The 2 degradation products of chlorpropamide, *p*-chlorobenzenesulfonylurea (II) and *p*-chlorobenzenesulfonamide (III), correspond to *p*-tolylsulfonylurea and *p*-tolylsulfonamide isolated by Mohnike *et al.*(4,5) from tolbutamide-treated dogs.

The recovery of 86% of the unchanged drug in the rabbit 24 hours after a single dose is evidence that chlorpropamide is resistant to metabolism in this species.

Johnson *et al.*(6), using S^{35} labeled chlorpropamide, reported no apparent metabolic alteration of the drug in humans. In this investigation of urinary excretion products following chlorpropamide therapy in man, it has been shown that metabolic degradation does occur. One metabolite has been isolated and identified as *p*-chlorobenzensulfonamide. It is possible that *p*-chlorobenzenesulfonylurea is also present but sufficient confirmatory data are not available. The paper chromatograms showed an additional metabolite that has not been identified. Excretion of chlorpropamide *per se* was proven by paper chromatography and infrared absorption spectra. Failure to get crystalline material was probably due to small amount of water in the eluate which caused degradation of chlorpropamide to sulfonamide during sublimation.

Summary. Metabolism of 1-(*p*-chlorobenzenesulfonyl)-3-n-propylurea was investigated in dog, rabbit and man. The rabbit excreted more than three-fourths of the drug without chemical alteration. Less than half of orally ingested drug was excreted unchanged by the dog, most of the remainder appeared as *p*-chlorobenzenesulfonylurea and *p*-chlorobenzenesulfonamide. As the result of a limited investigation in man, unchanged chlorpropamide and *p*-chlorobenzenesulfonamide were found in the urine. One other possible metabolite was indicated by paper chromatograms.

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Effect of Triiodothyronine and Cortisol on Intracellular Penetration of D-xylose. (25056)

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Studies with uncut rat diaphragm preparation demonstrated the *in vitro* effectiveness of insulin in permitting entry of D-xylose into a muscle fiber water space otherwise unavailable to sugar for distribution(1,2,3). To determine whether other hormonal treatments influence sugar penetration in this preparation, the effect of epinephrine, cortisol, and triiodothyronine (TIT) administered *in vitro* and *in vivo* have been examined. Since it was reported that metabolic poisons increase sugar penetration(2), it was of interest to learn if depletion of energy reserves *via* starvation would influence volume of distribution available to sugar.

Methods. The uncut rat diaphragm preparation and technics employed for pentose and sucrose determination were previously described(3). Incubation medium contained 128 meq/l-NaCl, 5.1 meq/l-KCl, 2.72 meq/l-$CaCl_2$, 1.02 meq/l-$MgSO_4$, 20 meq/l-$Na_2HPO_4/NaHPO_4$ buffer system (pH 7.2) and D-xylose at 4 mg/ml ± glucose at 1 mg/ml, or sucrose at 8 mg/ml. For *in vitro* experiments, TIT* was used as sodium salt (dissolved in 0.13 N-NaOH) at final concentration of 6 μg/ml medium. For *in vivo* administration, 300 μg TIT/day/rat were given ip 4 days. Epinephrine HCl was used *in vitro* at concentration of 2 μg/ml. Cortisol† was used *in vitro* at 40 μg/ml in 1% Tween 80 or *in vivo* at dosage regimen of 2 mg/day/rat ip. Insulin‡ was used at 0.4 units/ml (16 μg/ml) *in vitro.* Incubations were carried out in 50 ml medium volume at 38°C with shaking (90 cycles/min. amplitude 8 cm). Following incubation, diaphragm muscle was removed from rib cage, wiped across clean glass plate, weighed, and placed into a tube containing 2 ml distilled H_2O from which xylose or sucrose was extracted and determined. Sugar distribution in tissue was expressed relative to concentration in the medium. All animals were fed *ad lib.* except in experiments where 24 hr or 56 hr starvation period is indicated. Incubations were carried out for 60 and 90 minute periods since previous kinetic studies have shown that equilibration is attained in available water space within 60 minutes.

Results. Table I shows results obtained following *in vitro* or *in vivo* administrations of epinephrine, triiodothyronine, cortisol and/or insulin or starvation. These findings may be summarized as follows:

Triiodothyronine. Addition of TIT to incubation medium had no demonstrable effect upon distribution of D-xylose during 60 or 90 minute incubation. However, following parenteral administration of TIT, distribution of D-xylose is increased over controls. Treatment with TIT produced no change in total tissue water (79.1 ml/100 g ± 0.6) or in the sucrose space which may be assumed a measure of extracellular space. Thus increase in tissue xylose would seem to result from increased intracellular penetration. Furthermore, when diaphragms of TIT treated animals are incubated with insulin *in vitro,* the

* L-Triicdothyronine generously supplied by Dr. A. E. Heming, Smith, Kline & French Laboratory.

† Cortisol generously supplied by Dr. R. H. Silber, Merck Inst. for Ther. Res.

‡ Insulin was generously supplied by Dr. E. Rohrman, Eli Lilly & Co.