TABLE 2. THE BSP RETENTION VALUES (%) IN RATS, INTOXICATED WITH $CCl_4$ AND TREATED WITH FH 1

| No. of experiments | Denomination of group | | | | | |
|---|---|---|---|---|---|---|
| | control | ES | $CCl_4$ | ES | $CCl_4$ + FH 1 | ES |
| I | 14 | ±4 | 27 | ±6 | 19 | ±5 |
| II | 18 | ±3 | 36 | ±6 | 17 | ±1 |
| III | 18 | ±4 | 38 | ±4 | 18 | ±3 |

FH 1 was administered for 5 days after the last (6) injection of $CCl_4$. After this 5 days of treatment with FH 1, BSP (bromosulfophtalein) was administered all the groups i.v. in sol. 1% in amount of 5 mg/100 g A, blood samples was gathered in after 1 and 10 min. The quantity of BSP in serum was determined by the Shoemaker method and the retention values was calculated with the formula:

$$\frac{\text{Concentration BSP at 10 min}}{\text{Concentration BSP at 1 min}} \times 100$$

treatment the animals were sacrificed for microscopical and bromosulfophtalein (BSP) retention investigations. The results obtained are listed in Figs. 2 and 3 and Table 2.

The allyl alcohol intoxication is characterized by a perilobular liver necrosis. FH 1 diminishes the intensity of those lesions (Table 1 and Fig. 1). The administration the FH 1 in chronic liver damages induced by $CCl_4$ also re-establishes the metabolical function of the liver (BSP values return to normal—Table 1) and diminishes the centrolobular necrosis of hepatocytes (Figs. 2 and 3). On the basis of these results, it may be concluded that FH 1 is an efficient therapeutic agent in some forms of experimental liver damage.

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## The subcellular distribution of pancreatic kallikrein

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THE KALLIKREINS are a group of enzymes, probably isoenzymes, which occur in the submaxillary gland and the pancreas. Subcellular studies have shown that kallikrein occurs in granules which sediment at low *g* min values.[1–3] This finding has been recently confirmed.[4,5] In the present experiments the intracellular distribution of pancreatic kallikrein was studied and its distribution pattern compared with that of amylase and trypsin.

Cats and dogs, which had been allowed food *ad libitum*, were anaesthetised with intraperitoneal sodium phenobarbitone (45 mg/kg), exsanguinated and the pancreas removed, rinsed in 0·32M sucrose, dried on filter paper and weighed. The glands were cut into small pieces and homogenised

in 0·32M sucrose with an Ultra-turrax homogeniser. The mean concentration of fresh gland tissue was 71·3 mg/ml. The procedure for differential centrifugation was similar to that described previously.[2] Initial separation of the nuclear fraction was carried out on a B.T.L. centrifuge and the subsequent fractionation on an M.S.E. 65 centrifuge. The *g* min values used for the separation of particles are shown in Table 1: $P_1$, Nuclear fraction $8{\cdot}25 \times 10^2$; $P_2$ zymogen or heavy mitochondrial fraction $14{\cdot}25 \times 10^4$; $P_3$, lysosomal or light mitochondrial fraction $6{\cdot}15 \times 10^5$; $P_4$ microsomal fraction $8{\cdot}7 \times 10^6$.

TABLE 1. SUBCELLULAR DISTRIBUTION OF KALLIKREIN AND TRYPSIN IN CAT PANCREAS

| | | Kallikrein | | Trypsin* | | Potassium |
|---|---|---|---|---|---|---|
| Fraction | *g* min | PTR | RSA | PTR | RSA | PTR |
| $P_1$ | $8{\cdot}25 \times 10^2$ | 1·0 | 0·27 | 5·3 | 0·85 | 3·1 |
| $P_2$ | $14{\cdot}25 \times 10^4$ | 53·5 | 3·20 | 13·9 | 0·79 | 3·4 |
| $P_3$ | $6{\cdot}15 \times 10^5$ | 1·0 | 0·09 | 29·9 | 1·74 | 3·7 |
| $P_4$ | $8{\cdot}7 \times 10^6$ | 0·7 | 0·02 | 40·1 | 1·56 | 4·5 |
| Supernatant | | 43·2 | 1·28 | 10·7 | 0·33 | 85·1 |
| Total recovery (%) | | 157·1 | | 119·2 | | 115·2 |
| Number of experiments | | 3 | | 2 | | 2 |

PTR: percentage of total recovery. RSA: relative specific activity.
* Enterokinase activated trypsin activity.

For the kallikrein assay aliquots of subcellular fractions were diluted in either Mg-free Tyrode or 0·85% saline; the consequent change in osmolarity causes the rupture of membranes of subcellular organelles.[6] The substrate for kallikrein was prepared by heating dog plasma for 3 hr at 61° in order to destroy plasma kininase and kallikreinogen.[7] The substrate and enzyme were added without prior incubation to the isolated organ bath which had a constriction (1–2 mm internal diameter) at the base to restrict the incubation medium to a definite volume. The kallidin released by kallikrein from dog plasma was assayed on the isolated guinea-pig ileum suspended in Mg-free Tyrode solution at 35° and bubbled with 95% $O_2$–5% $CO_2$. The assays were carried in the presence of soya bean trypsin inhibitor ($5 \times 10^{-4}$ g/ml) and 1-cysteine ($2 \times 10^{-4}$ g/ml); the latter was used because it is an effective inhibitor of tissue peptidases which inactivate kallidin, and in addition it enhances the enzymatic activity of pancreatic kallikrein.[8] Mepyramine ($10^{-8}$ g/ml) and atropine ($10^{-8}$ g/ml) were also added to the bath fluid before each test. Freeze dried pancreatic tissue was dissolved in 0·85% saline (500 mg/ml), centrifuged and the supernatant assayed for 'active' kallikrein; the total kallikrein activity was determined by incubating the extract with enterokinase (10–50 μg/ml) and soya bean trypsin inhibitor (1·25 mg/ml) at 37° for 1 hr prior to assay. Although enterokinase activated kallikrein activity was measured in the subcellular fractions, no direct assay comparison was made with the non-activated fractions.

To measure total trypsin activity, subcellular fractions were preincubated with enterokinase (400 μg/ml) in Tris-HCl–$CaCl_2$ buffer (pH 7·4) at 37° for 1 hr; 0·25 ml of the incubation mixture was added to 0·1 ml 0·01M benzoylarginine-*p*-nitranilide (BAPA) in 2·65 ml Tris-HCl–$CaCl_2$ buffer (pH 7·4) at 37°. The rate of hydrolysis of BAPA was followed at 390 mU on a Unicam SP800. The measured trypsin activity in all fractions was abolished by soya bean trypsin inhibitor. Amylase was measured by the iodine titration method[2] and readings made on a Unicam SP1300 colorimeter. Protein[9] and potassium (the latter as a cytoplasmic marker) were also measured in the fractions.

The results (Table 1 and 2) show that kallikrein-containing granules mainly sediment at the *g* min value of $14{\cdot}25 \times 10^4$. Both the glandular kallikreins, submaxillary[1–3] and pancreatic, are therefore stored in granules which sediment at low *g* min values. Whereas submaxillary kallikrein occurs only in an active form pancreatic kallikrein may occur mostly in an inactive form. Studies on freeze-dried pancreatic gland tissue suggests that a significant amount of kallikrein is present *in situ* in an inactive form, the proportion varying from one species to another. The percentage of active kallikrein in freeze-dried pancreatic tissue was as follows: Cat 15 per cent (10 per cent, Professor E. Werle,

personal communication), rabbit 25 per cent, dog 30 per cent and rat 65 per cent. Since the kallikrein in the subcellular fractions was readily measurable, it was assumed that activation through autolysis had occurred during the experimental procedure. However, the pattern of intracellular distribution of kallikrein was unaffected when the fractions were assayed after incubation with enterokinase.

The subcellular distribution of trypsin in the cat and dog was different from that of kallikrein (Table 1 and 2) but identical to that described by Siekevitz and Palade[10] in the pancreas of guinea-pigs

TABLE 2. SUBCELLULAR DISTRIBUTION OF KALLIKREIN, TRYPSIN AND AMYLASE IN DOG PANCREAS

| | | Kallikrein | | Trypsin* | | Amylase | | Potassium |
|---|---|---|---|---|---|---|---|---|
| Fractions | *g* min | PTR | RSA | PTR | RSA | PTR | RSA | PTR |
| $P_1$ | $8{\cdot}25 \times 10^2$ | 0·8 | 0·21 | 2·8 | 0·90 | 0·15 | 0·03 | 2·3 |
| $P^2$ | $14{\cdot}25 \times 10^4$ | 47·3 | 2·47 | 19·3 | 1·00 | 10·9 | 0·57 | 4·3 |
| $P_3$ | $6{\cdot}15 \times 10^5$ | 5·8 | 0·34 | 13·1 | 0·74 | 1·2 | 0·06 | 5·0 |
| $P_4$ | $8{\cdot}7 \times 10^6$ | 2·0 | 0·07 | 43·2 | 1·52 | 3·4 | 0·12 | 7·1 |
| Supernatant | | 43·3 | 1·36 | 27·5 | 0·88 | 84·2 | 2·71 | 81·0 |
| Total recovery (%) | | 105·8 | | 156·4 | | 157·3 | | 112·0 |
| Number of experiments | | 2 | | 2 | | 2 | | 2 |

PTR: percentage of total recovery. RSA: relative specific activity.
* Enterokinase activated trypsin activity.

starved for 48 hr and then fed for 1 hr before removal and fractionation of the gland. The distribution of amylase in dog pancreas, however, was qualitatively similar to that of kallikrein (Table 2); the percentage recovery of amylase in the various fractions was quantitatively similar to that found in the submaxillary gland of the guinea-pig.[1-2] Amylase in the subcellular fractions of cat pancreas was not measured because of the low activity in the gland; amylase activity was about twenty times less than that in dog pancreas and about forty times less than that in guinea-pig submaxillary gland.

These preliminary data on the intracellular distribution of kallikrein, trypsin and amylase do suggest that these enzymes are probably sequestered in separate storage granules with perhaps unit membranes of differing chemical composition. The localisation of kallikrein in organelles similar to zymogen granules in the pancreas is of physiological importance in view of the fact that kallikrein in the cat submaxillary gland[11] and pancreas[12] is considered to have a local endocrine role; the present and previous[1-3,13] results however indicate that kallikrein is stored in the type of granules which are known to discharge into acinar ducts and not undergo reverse exostosis and empty into the interstitial tissue space.

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## S-methylation, oxidation, hydroxylation and conjugation of thiophenol in the rat

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THIOPHENOL is an important chemical intermediate utilized in the manufacture of lubricating oil additives, antioxidants, resins, insecticides, herbicides and pharmaceuticals.

Parke[1] reported that rabbits excreted no free thiophenol after its oral administration, but instead the urinary products were thiophenyl glucuronide and, possibly, phenylthiosulfuric acid. Oxidation of thiophenol to diphenyldisulfide (DPDS) by spores of the fungus, *Myrothecium verrucaria*, was reported by Mandels.[2] Recently, Gessner and Acara[3] described the S-glucosylation of thiophenol in four insect species *in vivo* and *in vitro*. In this communication we present evidence indicating a third, major biotransformation pathway for thiophenol in the rat.

Thiophenol-$^{35}$S (PSH), 14·7 mc/m-mole, from the Radiochemical Centre, Amersham, England, was of a radiochemical purity of greater than 98 per cent as determined by gas chromatography. Methylphenylsulfide-$^{35}$S (MPS) and methylphenylsulfone-$^{35}$S ($MPSO_2$) each with a specific activity of 1·13 mc/m-mole, from Stauffer Chemical Company, Western Research Center, Richmond, Calif., were radiochemically pure samples after purification by preparative-scale thin-layer chromatography (TLC). Authentic unlabeled samples were from the following sources: PSH from Pitt-Consol, Newark, N.J.; MPS and $MPSO_2$ from Aldrich Chemical Company, Milwaukee, Wis.; methylphenylsulfoxide (MPSO) and DPDS from Stauffer Chemical Company, Richmond, Calif.

Four rats of the Long–Evans strain (two males and two females, 116–124 g) were orally dosed with 6 mg/kg of PSH-$^{35}$S. Each of two male rats weighing 280–300 g was similarly treated with 2·5 mg/kg of either MPS-$^{35}$S or $MPSO_2$-$^{35}$S. The treated rats were housed in glass metabolism cages.[4] Urine samples were collected for 60 hr in the case of PSH-$^{35}$S and for 64 hr in the case of MPS-$^{35}$S or $MPSO_2$-$^{35}$S. Nonradioactive PSH (200 $\mu$g) was added to 70 ml of freshly collected urine from untreated rats and processed in the same manner as the experimental urine, thus serving as a control. Urine from each group was pooled and extracted twice with benzene to separate the polar (aqueous phase) and nonpolar (benzene phase) metabolites. Polar fractionated urine samples were hydrolyzed by refluxing for several hours with 3 N $H_2SO_4$. Hydrolyzed radioactive products were extracted with diethyl ether for subsequent chromatography.

TLC analysis of the benzene-soluble and water-soluble metabolites, before and after hydrolysis, involved Silica gel H (250 $\mu$ thick) precoated plates (Analtech, Inc., Wilmington, Del.) and chloroform:ethyl acetate(1:1) mixture (CEA) for development. $R_f$ values in this system were as follows: PSH,0·95; MPS, 0·90; $MPSO_2$, 0·83; MPSO, 0·34. The benzene-soluble metabolite and $MPSO_2$ were chromatographed in two additional solvent systems: benzene: diethyl ether(7:3) mixture (BE) and *n*-hexane:chloroform(3:2) mixture(HC). Water-soluble metabolites were also resolved on Silica gel F-254 (250 $\mu$ thick) precoated plates (Brinkmann Instrument Company, Inc., Westbury, Long Island, N.Y.) using *n*-butanol:acetic acid:water(2:1:1) mixture (BAW). They were also examined before hydrolysis in the CEA system. Radioactive products were detected by autoradiography using