

Chlorine dioxide retards the chlorine oxidation of all the compounds in Table 1; in some cases, however, the retardation was rather weak. The oxidations of the methyl glucosides, cellobiose, and cellobiitol are strongly retarded and that of gluconic acid is weakly retarded. The other compounds lie in between these extremes. The amount of retardation may indicate the degree by which the carbohydrate reacts by a radical mechanism.

The reaction of chlorine, under these conditions, with some simpler substances, e.g. dioxane, was also found to be retarded by chlorine dioxide.

The mechanism of the initiation of the dark reaction is obscure. Walling and Mintz have recently shown that molecule induced homolyses take place readily between some aldehydes and ethers, and *t*-butyl hypochlorite.<sup>15</sup> An analogous mechanism involving the substrate and chlorine or hypochlorous acid could be suggested for the present reaction. A radical chain could then be considered to be carried by chlorine atoms or, less probably, hydroxyl radicals. It is known that chlorine dioxide, at least in the gas phase, reacts with chlorine atoms.<sup>14</sup>

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## The Zymogen of Phospholipase A<sup>2</sup> in Rat Pancreatic Juice

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The presence of phospholipase A<sup>1</sup> and A<sup>2</sup> activities in pancreatic tissue, pancreatic juice and duodenal contents is well established.<sup>1-4</sup> Porcine pancreatic lipase has been shown to hydrolyse lecithin, preferentially at the  $\alpha$ -fatty acid ester linkage.<sup>5</sup> Recently, de Haas<sup>6</sup> has presented evidence for the existence of precursor of phospholipase A in porcine pancreas. This "prophospholipase", subjected to tryptic digestion can be converted to its active form by splitting off a heptapeptide.

This investigation was undertaken in order to determine whether this zymogen form of phospholipase also exists in pancreatic juice and the relationship of its active form to other phospholipid splitting enzymic activities in rat pancreatic juice. Pancreatic juice from 3 to 4 male Sprague-Dawley rats was collected into a container chilled with dry ice after cannulation of the pancreatic ducts. The juice was subsequently lyophilised and used in this study within one week. The experiments were performed with the powder dissolved in ice cold water to the original concentration. Phospholipase, lipase and activity against micellar monoglyceride were assayed as previously described,<sup>2,7</sup> with the exception that the incubation period for phospholipase assay was shortened from 2 h to 10 min. Tryptic digestion was carried out as follows:

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0.2 ml of enzyme source was diluted with 0.8 ml of a solution containing 20  $\mu\text{g}$  of trypsin in 0.1 M Tris-HCl buffer, pH 7.6, 0.02 M with respect to  $\text{CaCl}_2$ . This mixture was preincubated at 37° for 10 min, and was then used for estimation of enzymic activities. Previous observations had shown that under these conditions complete activation of phospholipase occurred within 10 min.

The activities of specimens of lyophilized pancreatic juice against micellar lecithin were found to vary considerably, but it seemed that the most recently prepared powders of rat pancreatic juice contained the smallest amounts of free phospholipase activity. When digested with trypsin, however, the phospholipase activity of whole pancreatic juice, as measured by the release of fatty acid from micellar lecithin, is increased by approximately 10 times.

As it was possible that several enzymic activities residing in whole rat pancreatic juice are responsible for this hydrolysis, fractionation of the juice by gel filtration was employed in order to distinguish these

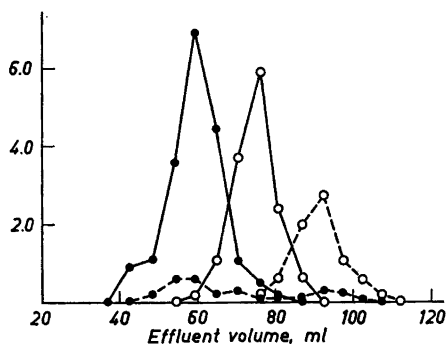


Fig. 1. Chromatography of lyophilized pancreatic juice on Sephadex G-100. Temp. 4°. Eluted with 0.15 M NaCl. Flow rate approx. 8 ml/h. Void volume 40 ml; ●—● activity against micellar monoolein; ○—○ activity against emulsified triglyceride; ●---● activity against micellar lecithin; ○---○ activity against micellar lecithin after tryptic digestion of the enzyme source. Incubation times, mono-glyceride 60 min at room temperature; tri-glyceride, 5 min at room temperature; lecithin 10 min at 37°. Ordinates:  $\mu\text{equiv.}$  fatty acid released/min per fraction from micellar lecithin. The activities against emulsified triglyceride and micellar monoolein are given in arbitrary units in order to indicate the elution volumes of these enzymes.

activities. For this, Sephadex G 75 and G 100 (Batch Nos. To — 79; To — 5218) were used. Fig. 1 shows the distribution of hydrolytic activities against micellar lecithin assayed at pH 9.0, micellar monoolein and emulsified triolein, both assayed at pH 5.8, in such a separation. It can be seen that the three groups of fractions show activity against the lecithin substrate at pH 9.0 as measured by the release of fatty acid. In only one of these, the fractions corresponding to a  $K_{av}$  of approximately 0.60 on Sephadex G 100, could the activity be increased by tryptic digestion. This activity could be increased by a mean of 12 times (6 expts.). The lipolytic enzyme attacking emulsified triolein was unaffected by tryptic digestion carried out under the same conditions, as judged by its ability to release fatty acid from the triglyceride.

In order to study the positional specificity of the three enzymic activities,  $\beta$ - $^3\text{H}$  acyl-lecithin was used as the substrate. This was prepared by the method of Bennett and Tattrie,<sup>8</sup> and was mixed with egg lecithin. The same incubation conditions were used as described above, except that the enzymic hydrolysis of the lecithin substrate was allowed to continue for 2 h. The preincubations with trypsin were performed as above. Table 1 gives the specific activities (counts/min/ $\mu\text{mole}$  of fatty acid) of the fatty acids released during digestion by the three enzymic activities. These specific activities were compared with the specific activity of the fatty acid in the  $\beta$ -position of the lecithin substrate as measured by hydrolysis of this substrate with *Crotalus adamanteus* venom using the method described by Hanahan.<sup>9</sup> These relative specific activities are then a measure of the proportion of the released fatty acid derived from the labelled fatty acids in the  $\beta$ -position of the lecithin. The figures show that the enzyme having a  $K_{av}$  of 0.60 preferentially releases the fatty acids of the  $\beta$ -position of lecithin, and can therefore be regarded as phospholipase A activity while a much smaller proportion of the  $\beta$  fatty acids is liberated by the other two enzymes. However, there is still a hydrolysis of alpha fatty acid in the phospholipase area which might be due to contamination of these fractions by lipase. In order to study the isolated phospholipase activity, whole pancreatic juice was subjected to heating for 10 min at 70° at pH 4.0 in 0.1 M citric acid-phosphate buffer. This treatment was shown to destroy all enzyme activity against emulsi-

*Table 1.* Specific activities of fatty acids released from  $\beta$ - $^3\text{H}$  acyl-lecithin by the action of pancreatic juice proteins separated on Sephadex G-100. 20 mg lyophilized rat pancreatic juice was dissolved to the original volume and separated by gel filtration on Sephadex G-100 with 0.15 M NaCl as the eluent. The obtained fractions were incubated with emulsified triolein, micellar monoolein and, after tryptic digestion, with micellar lecithin. Fractions with maximal lipolytic activities against the three substrates were then used for incubation with  $\beta$ - $^3\text{H}$  acyl-lecithin. The specific activity (cpm/ $\mu\text{equiv.}$ ) of the fatty acids released was compared to the specific activity of the fatty acid at the  $\beta$ -position of the lecithin which was determined by the method of Hanahan using *Crotalus adamanteus* venom.

Enzyme source	Specific activity of released fatty acid cpm/ $\mu\text{equiv.}$	Relative specific activity
Peak at $K_{\text{av}}$ 0.22	2829	0.470
Peak at $K_{\text{av}}$ 0.37	1894	0.314
Peak at $K_{\text{av}}$ 0.60	4892	0.813
<i>Crotalus adamanteus</i> venom	6020	1.000

fied triolein and micellar monoolein while activity against micellar lecithin was virtually unchanged. This enzyme preparation was used both with and without predigestion with trypsin to hydrolyse the labelled substrate and its activity compared with snake venom hydrolysis of this same substrate. It was found that the specific activity of the fatty acid released by the phospholipase preparation from pancreatic juice was as great as that released by *Crotalus adamanteus* venom; this proves that the phospholipase activity of pancreatic juice which can be activated by tryptic digestion is exclusively a phospholipase A<sup>2</sup>. The specific activities of the fatty acids released from micellar lecithin incubated with trypsin activated whole pancreatic juice and incubated with trypsin activated pancreatic juice previously heated at 70° for 10 min were very similar. This means that after tryptic digestion under the incubation conditions, pH 9.0 at 37°, the predominant enzymic activity in pancreatic juice against micellar lecithin is due to a phospholipase A<sup>2</sup>.

These results indicate that rat pancreatic juice contains a small and variable amount of free phospholipase activity while a much larger amount can be released from a zymogen by a short period of tryptic digestion. The amounts of trypsin that must be used to achieve complete activation must exceed the trypsin inhibitory activities of the juice and therefore it can be expected that the amounts of trypsin required for complete activation of the phospholipase

zymogen in whole pancreatic juice would be greater than these required for activation of the partially purified proenzyme obtained by gel filtration. In the latter case the trypsin inhibitory activity is separated by reason of the smaller molecular weight of this substance. In one experiment, this inhibitor was measured according to the method described by Arnesjö and Grubb<sup>10</sup> and found to be eluted with a  $K_{\text{av}}$  of 0.64 on gel filtration with Sephadex G 75; this corresponds to a molecular weight of approximately 7000. It is possible that enzymic activities against the alpha fatty acid of lecithin can be ascribed to the action of the two enzymes active against micellar and emulsified glycerides described above. The detection of a zymogen form of phospholipase A in the pancreatic tissue<sup>6</sup> and in pancreatic juice could explain the differences in the activities measured in pancreatic juice and pancreatic tissue on the one hand and duodenal contents on the other.<sup>1-4</sup> Hydrolytic activity towards lecithin in duodenal content must be relatively high as Borgström has shown that there are considerable quantities of lysolecithin in the contents of the upper small intestine.<sup>11</sup>

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## Lattice Parameters of the CuAu(I) Phase

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Despite the many studies of Cu-Au alloys, it seems that the lattice dimensions of the CuAu(I) phase have never been measured continuously as a function of composition. The composition dependence of these parameters is of interest, however, since a change in the slope of  $a$  and  $c$  versus composition at  $\sim 50$  atomic % is expected on the basis of the corresponding data for the isostructural phases in the Ir-Mn, Ni-Mn, Pd-Mn, and Pt-Mn systems.<sup>1-4</sup>

The ordering in the CuAu(I) type structure establishes alternate layers of Cu and Au atoms normal to the unique axis, and atomic contacts within the layers occur along [100] and [010] of the primitive cell containing two atoms. The filling of one layer with the larger Au atoms as the Au content of the phase increases towards

50 atomic %, must accordingly cause the  $a$  parameter to increase. When the Au layer is filled at 50 atomic %, we expect the  $a$  spacing to be about equal to the atomic diameter of pure Au. Beyond 50 atomic % Au, when Au starts to enter the Cu layer, interlayer Cu-Au contacts are replaced by Au-Au contacts, causing the  $c$  parameter to expand (since  $a$  has already expanded to accommodate the Au-Au contacts). These compulsory expansions of  $a$  below 50 atomic % Au and of  $c$  above 50 atomic % Au control the dimensions of the unit cell, and the variations of  $c$  below and of  $a$  above 50 atomic % Au only keep the appropriate volume for the unit cell. These conditions must cause a change of slope in the lattice parameter curves at the equiatomic composition.

*Experimental.* Alloys ranging from 40 to 60 atomic % Au at intervals of 2 atomic % were prepared by melting weighed quantities of 99.99 + % pure Cu and Au in evacuated, sealed silica (clear) tubes. The alloys were annealed for 6 h at 650°C (to reduce the number of nuclei for ordering and hence to increase the ordering rate<sup>5</sup>) and then annealed at 300–310°C for more than 16 months, with intermittent cold work treatments, including the preparation of filings and X-ray examination. Nominal compositions were considered sufficiently accurate.

X-Ray powder photographs were taken with CuK $\alpha$ -radiation in a 190 mm diameter Unicam camera. The lattice constants were determined from the high-angle reflections by a Nelson-Riley<sup>6</sup> type extrapolation. Guinier photographic data of all alloys were collected with a 80 mm diameter camera using monochromatized CuK $\alpha_1$ -radiation ( $\lambda = 1.54050 \text{ \AA}$ ) and KCl as internal standard. The lattice constants calculated from the latter data were refined by applying the method of least squares. Unit cell dimensions determined by these two methods agreed within  $\pm 0.001 \text{ \AA}$ .

*Results.* The CuAu(I) phase appears to have a homogeneity range between 46 and 54 atomic % Au for alloys annealed at  $\sim 300^\circ\text{C}$ . Alloys from 40 to 44 and from 56 to 60 atomic % Au contained the CuAu(II) phase or mixtures of phases (CuAu(I), CuAu(II), and CuAu<sub>3</sub>), in fair agreement with the data given in Hansen<sup>7,8</sup> and/or Pearson.<sup>9,10</sup>

The measured lattice parameters of the CuAu(I) phase (Fig. 1) do indeed show the predicted change in slope at 50 atomic % Au, but this is not apparent in the unit

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