Both salivary and serum amylase determinations were carried out by the method of Van Loon (*5*) (Van Loon's amylase units are numerically equal to Somogyi amylase units), using 1/100 dilutions of serum and 1/20,000 dilutions of saliva instead of the usual 1/10 dilution that is recommended for human serum. In an earlier study in this laboratory, human salivary amylase levels were found to be 50,000 to 200,000 units/100 ml, and 1/5000 dilutions of saliva were appropriate in the analyses. Incidentally, no diastaticlike action of pilocarpine (*6*) on starch or other interference with salivary amylase determination was noted.

The data in Table 1 show that there are wide variations in rat salivary amylase levels, but it is apparent that the average (626,000) is still several times that in human saliva. Thus, contrary to previous statements (*2*), man does not have "the highest ptyalin concentration of all animals."

These data on rats were of interest in view of the report (*7*) that in depancreatized rats, serum amylase levels and urinary amylase excretion remain essentially the same as in normal rats. Thus, some organ or organs other than the pancreas must be a source of rat serum amylase. The salivary glands might conceivably be such a source. However, comparison of salivary and serum amylase levels in rats showed little correlation. Also, in a rat that was sacrificed in this laboratory on the seventh day after surgical removal of its salivary glands, the serum amylase fell only slightly. The serum amylase preoperatively was 2500; 7 days postoperatively, it was still 1800. Since, with no saliva, the rat experienced some difficulty in eating, the fall in serum amylase level could be explained on a purely nutritional basis. Saline washings of this rat's mouth showed no trace of amylase activity.

Table 1. Salivary and serum amylase in rats.

| Amylase (Van Loon units/100 ml) | | Salivary/serum |
|---|---|---|
| Salivary | Serum | |
| 1,340,000 | 4280 | 312 |
| 1,300,000 | 4810 | 270 |
| 1,180,000 | 4070 | 290 |
| 1,000,000 | 4070 | 245 |
| 750,000 | 4150 | 181 |
| 670,000 | 4230 | 158 |
| 630,000 | 3660 | 172 |
| 617,000 | 4400 | 140 |
| 510,000 | 3310 | 154 |
| 361,000 | * | |
| 307,000 | 3740 | 82 |
| 247,000 | 3660 | 67 |
| 205,000 | 4060 | 50 |
| 181,000 | * | |
| 91,000 | 3740 | 24 |

* Samples lost.

As one might have predicted, the amylase in rat saliva is apparently an α-amylase. Its action on soluble starch produced the progressive changes to the amylo-, erythro- and achrodextrin stages (as followed by the iodine reaction) that are typical of the action of α-amylase but not that of β-amylase.

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## Synthesis of 5-Hydroxypipecolic Acid and Separation of Its Diastereoisomers

The metabolic and structural relationships between ornithine and proline (*1*), hydroxyornithine and hydroxyproline (*2*), and lysine and pipecolic acid (*3*) prompted us to convert δ-hydroxy-L-lysine to 5-hydroxypipecolic acid, the homolog of hydroxyproline. This amino acid has recently been isolated from *Rhapis flabelliformis* (*4*) and has been obtained as an intermediate in the synthesis of baikiain (*5*).

δ-Hydroxy-L-lysine containing 18 percent *allo*hydroxy-D-lysine (*6*) was treated with 1.5 equivalents of nitrosyl chloride in 6*N* hydrochloric acid and kept at 60°C for 25 minutes. The reaction mixture was assayed for total nitrogen (Kjeldahl) and for α-amino nitrogen by evolution of carbon dioxide after oxidation with chloramine-T (*7*). It was found that about one-half of the total nitrogen was present as α-amino nitrogen. Assuming little or no formation of the α,ε-dichlorocaproic acid, it follows that approximately equal amounts of the α-chloro and ε-chloro acids were formed. The mixture was cyclized by bringing the aqueous solution to a *p*H of 11 with barium hydroxide and warming on the steam bath for 10 minutes.

The products of this reaction were studied by ion-exchange chromatography in an attempt to separate the diastereoisomers. The methods were patterned after those of Moore and Stein (*8*). A 150- by 0.9-cm column of Dowex 50-X8, 200 to 400 mesh (rescreened, wet, through 200 mesh), operated at room temperature and 4 ml/hr, was employed. Elution was started from a mixing chamber containing 130 ml of 0.3*M* citrate buffer (0.2*N* in sodium citrate) at *p*H 3.00. The *p*H was gradually increased by adding 0.2*N* sodium citrate to the buffer at a rate equal to one-half the elution rate. Buffer and citrate solution contained 1 percent of a detergent solution (*8*). One-milliliter fractions were collected. 5-Hydroxypipecolic acid was determined (*9*) by adding 1 ml of 2-percent ninhydrin in glacial acetic acid and placing the tubes in a boiling water bath for 15 minutes. After the mixture had been diluted with 5 ml of acetone-water (50/50 by volume), the concentration was determined from the absorbancy at 353 mμ.

Figure 1 shows a portion of the effluent curve that was obtained by chromatography of the reaction mixture from the cyclization of hydroxylysine. By admixture of an authentic sample of 5-hydroxy-L-pipecolic acid obtained from dates (*9*) or material kindly provided by A. I. Virtanen (*4*), the faster moving peak was identified as the natural isomer. The other peak was presumably the *allo* form since it showed an absorption spectrum after reaction with ninhydrin in glacial acetic acid identical with that obtained with the natural isomer. This was the same order of appearance as that observed for the diastereoisomers of hydroxyproline (*10*) on an ion exchange column, and it may be taken as a suggestion of the *trans* arrangement for the functional groups of 5-hydroxy-L-pipecolic acid. The yield from the cyclization reaction was only 2 to 5 percent.

Paper chromatography was also used to characterize the products of the cycli-

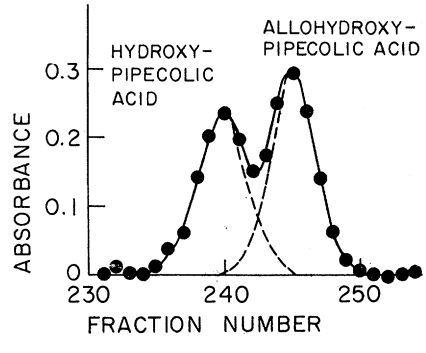


Fig. 1. Ion-exchange chromatography of the products of cyclization of δ-hydroxylysine showing the separation of the diastereoisomers of 5-hydroxypipecolic acid.

zation reaction. The methods described by Irreverre and Martin (*11*) were employed. Authentic 5-hydroxy-L-pipecolic acid gave a spot just to the left of proline (refer to Fig. 5A, of Irreverre and Martin, *11*). The *R* value (relative to alanine) was 1.31 in *t*-amyl alcohol-2,4-lutidine. The ninhydrin color was bright purple, fading to yellow over several days. Under ultraviolet light, it fluoresced bright cherry-red, or reddish-orange at lower concentrations. A greenish-blue color was produced with isatin. The reaction mixture from the cyclization of hydroxylysine showed this same spot. Another spot, identical in every respect, appeared slightly lower on the chromatogram. The *R* value was 1.00 in the basic solvent. It seemed probable that the diastereoisomers had separated on the paper. This hypothesis was tested by chromatographing hydroxy-L-proline and *allo*hydroxy-D-proline. These diastereoisomers separated to approximately the same degree and in the same direction. The *R* values were 1.04 and 0.83 in the basic solvent. Also, authentic 5-hydroxy-L-pipecolic acid showed only the lower spot after inversion to *allo*hydroxy-D-pipecolic acid. The inversion was accomplished by refluxing with acetic anhydride containing acetic acid (*12*) and subsequent hydrolysis.

A large, immobile spot was also present on the paper chromatograms of the cyclization mixture. This probably represented linear polymerization of the monochloro acids which could have accounted for the low yield of 5-hydroxypipecolic acid. Studies on the stereochemistry of 5-hydroxy-L-pipecolic acid are in progress.

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## Effects of Intravenously Administered Fat on the Serum Lipoproteins

It has been shown in our laboratories that daily intravenous infusions of a fat emulsion cause a significant decrease in the elevated serum lipid values of patients with idiopathic hyperlipemia or primary hypercholesteremic xanthomatosis (*1*). In order to study these effects in more detail, electrophoretic analyses of serum were carried out in eight normal human beings and in eight dogs before and after an intravenous infusion of emulsified fat (*2*).

The human beings were given a single intravenous infusion of an emulsion containing in a total volume of 500 ml either 50 g of cottonseed oil or 50 g of synthetic triolein, with soybean phosphatide, pluronic, and dextrose added as emulsifying and stabilizing agents (*3*). The dogs weighed about 15 kg and were given 300 ml of this emulsion. The time for the infusion averaged 4 hours in the human beings and 2 hours in the dogs. Blood samples were taken before and at the end of the infusion as well as for several hours afterward. Samples of serum were analyzed by paper electrophoresis as described previously (*4*).

In the eight human beings, the paper strips stained for lipids showed, prior to the infusion, two bands: (i) an intensely stained band in the beta globulin area, corresponding to the beta lipoproteins, and (ii) a less intensely stained band intermediary between alpha-1 globulin and albumin, corresponding to the alpha lipoproteins. At the end of the intravenous infusion, the beta lipoprotein band showed a densely stained trail extending back to the starting line, representing the chylomicrons. In addition, both lipoproteins moved faster: the beta lipoproteins moved either with a speed intermediary between beta globulin and alpha-2 globulin or with a speed of alpha-2 globulin (Fig. 1). The alpha lipoproteins in some subjects were split into two components, the larger component migrating with the speed of albumin and the other smaller component migrating ahead of albumin; in other subjects, all alpha lipoproteins migrated together as a broad band ahead of albumin. On the corresponding protein-stained paper strips, small amounts of protein were seen ahead of albumin, representing the protein moiety of the alpha lipoproteins. The increase in the electrophoretic mobility of the alpha and beta lipoproteins persisted for about 2 hours after the end of the infusion.

In the eight dogs, fasting serum samples showed on the lipid-stained paper strips an intensely stained band in the area between albumin and alpha-1 globulin, representing the alpha lipoproteins. In five dogs, small amounts of beta lipoproteins were present in the beta globulin region; in three dogs, no visible amounts were present. At the end of the infusion, the lipid-stained paper strips showed a densely staining trail extending to the starting line, representing the chylomicrons. In those dogs in which beta lipoproteins had been present in visible amounts prior to the infusion, they then migrated with increased speed in the region of alpha-2 globulin. The alpha lipoproteins were found in all instances ahead of albumin as a broad band on both the lipid- and the protein-stained paper strips.

So far, we have not been able to demonstrate electrophoretic changes in the serum after oral feeding of the fat emulsion, possibly because the concentration of lipids in the serum of the test persons did not rise as high as it did after the intravenous infusion.

The importance of the observed electrophoretic changes following the infusion of the fat emulsions lies in the fact that the changes are identical with those induced by the intravenous injections of heparin in normal persons during alimentary hyperlipemia (*4, 5*) and in patients with idiopathic hyperlipemia even when fasting (*4, 6*). In addition, in patients with idiopathic hyperlipemia, an injection of heparin had caused a de-

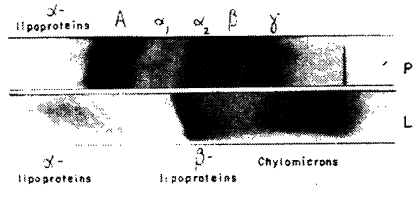


Fig. 1. Paper electrophoretic pattern of human serum obtained at the end of an intravenous infusion of fat. Upper half of the strip is stained for proteins (*P*), lower half is stained for lipids (*L*). On the lipid-stained paper strip, the beta lipoproteins, normally present in the beta globulin area, move now with the speed of alpha-2 globulins. The alpha lipoproteins, normally present in the area between albumin and alpha-1 globulins, move now ahead of albumin. On the protein-stained paper strip (*P*), small amounts of protein migrate ahead of albumin, representing the protein moiety of the alpha lipoproteins.