insulin hypoglycemia also seems supported by the observations that in obese subjects, in whom fasting NEFA levels are consistently elevated(18) and growth hormone levels are low(19), further fasting or hypoglycemia fails to increase the fasting value of growth hormone(19). The close interrelationship between NEFA plasma levels and the secretion of growth hormone deserves close attention even if, in this regard, other indices of the metabolic action of growth hormone are investigated.

Summary. Pituitary content of growth hormone was determined in rats submitted to insulin hypoglycemia, pretreated or not treated with bovine growth hormone. Exogenous growth hormone was capable of inhibiting endogenous growth hormone release, suggesting the existence of an "auto" feed-back mechanism. A close interrelationship was also observed between plasma NEFA levels and growth hormone secretion.

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Electrophoretic Behavior of Serum Amylase in Various Mammalian Species.* (31385)

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At least 30 enzymes are now recognized to exist in multimolecular forms. Such polymorphism may reflect differences in the evolutionary development of certain functionally related proteins. Attempts have been made to extend the concept of enzyme multiplicity to include amylase in human serum, but these have met with only limited success to date (1,2). It seems reasonable to presume that the starch-hydrolyzing activity in the serum of man as well as other species is derived from various tissues which have been demonstrated to contain amylase. With a view toward broadening our fundamental knowledge of the

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possible tissue or species specificity of the starch-hydrolyzing activity in the systemic circulation, the electrophoretic patterns of serum amylase were examined in 11 different mammals including man. All of the species examined possess pancreatic amylase activity, but several animals were selected for study that are known to lack this enzyme in saliva.

Materials and methods. Rat, dog, pig, horse, cow, cat, sheep, goat, guinea pig, rabbit and human serum were placed on Whatman 3MM filter paper strips measuring 6 × 35 cm. Volumes of either 0.1 or 0.2 ml of serum were used for this purpose depending on the serum total amylase activity. Electrophoretic separations were performed in a horizontal cell employing Veronal buffer (pH 8.6, $\mu = 0.075$) at constant potential of 80 volts for a period of 16 hours. A segment 3 cm in width was cut longitudinally from each strip, heat-fixed, and stained for protein. The unstained portion of each strip was then cut serially into 0.5 cm. segments which were individually extracted with 0.85% NaCl. The amylase activity of each eluate was measured saccharogenically in terms of the dinitrosalicylic color reaction(3).

Results. Monomorphic patterns of amylase were obtained by subjecting sera from sheep, rabbit, cat, guinea pig, horse and man to paper electrophoresis (Fig. 1). The single well-defined area of starch hydrolytic activity occupied a zone near the point of specimen application in all of these species, except the horse. In the latter animal, amylase activity was recovered from an electrophoretic region intermediate in position between albumin and the first globulin fraction. The bands of saccharogenic activity were relatively narrow in rabbit, guinea pig, cat and human serum, whereas a somewhat wider electrophoretic dispersion was obtained with specimens from the horse and sheep. Worthy of special note is the observation that a component of serum amylase in several of the species migrated cathodally from the origin in an area that was virtually devoid of stainable quantities of protein.

Polymorphic patterns consisting of at least 2 areas of sacchrogenic activity were demonstrable on electrophoretic strips prepared with pig, goat, rat, dog and cow serum (Fig. 2). In all cases, at least 1 amylase zone was located at or near the electrophoretic origin. However, only in dog serum did this almost immobile fraction account for a clear majority of the serum total amylase activity. Pig, goat and cow serum showed appreciable percentages of amylase with a mobility similar to that of albumin. Amylase in cow and goat serum was not sharply resolved into specific electrophoretic zones, while that of pig serum occupied 3 rather distinct positions.

Discussion. Of the various species studied, 4 exhibited electrophoretic patterns like that of man with a majority of amylase residing in the γ-globulin position. Amylase in horse serum also showed a single electrophoretic zone of activity, but this migrated anodally with a speed approximating that of albumin. Apparently, the amylase fraction in horse serum is more highly negatively charged than the corresponding enzyme fraction in man, rabbit, sheep, cat or guinea pig.

Failure of amylase to resolve into distinct fractions with paper electrophoresis does not necessarily imply that the enzyme exists as a single molecular form. Rabbit serum amylase, for example, has been separated into several saccharogenic fractions by means of DEAE-cellulose chromatography(4). Likewise, the γ-globulin zone amylase in normal human serum may be partitioned into at least 2 distinct fractions by electrophoresis on polyacrylamide gel(5).

The polymorphic electrophoretic patterns of serum amylase in the pig, rat, dog, goat and cow shared the feature of having at least 1 fraction of saccharogenic activity in the γ-globulin zone. A portion of this enzymatic material residing in or near the electrophoretic origin may derive from the pancreas. Supporting this view is the fact that crystalline porcine amylase when subjected to paper electrophoresis occupies the γ-globulin position(6). Previous studies(7) in dogs have also shown that removal of the pancreas diminishes the serum amylase in the γ-globulin area, but leaves that in the α- and β-globulin area virtually unaffected. Likewise, pancreatectomy in man diminishes the serum amylase activity in the γ-globulin zone. Se-

rum amylase in the γ-globulin area, as well as in the other electrophoretic regions of the pig, cow, dog, and rat, may be derived, in part, from the salivary glands. This, presumably, is not the case in the goat, which lacks salivary gland amylase. The dog and the cat are both carnivores, but the salivary glands of the latter animal are devoid of amylolytic activity. This may partially account for the difference in the patterns of saccharogenic activity of these two species.

The function of amylase in serum is ob-

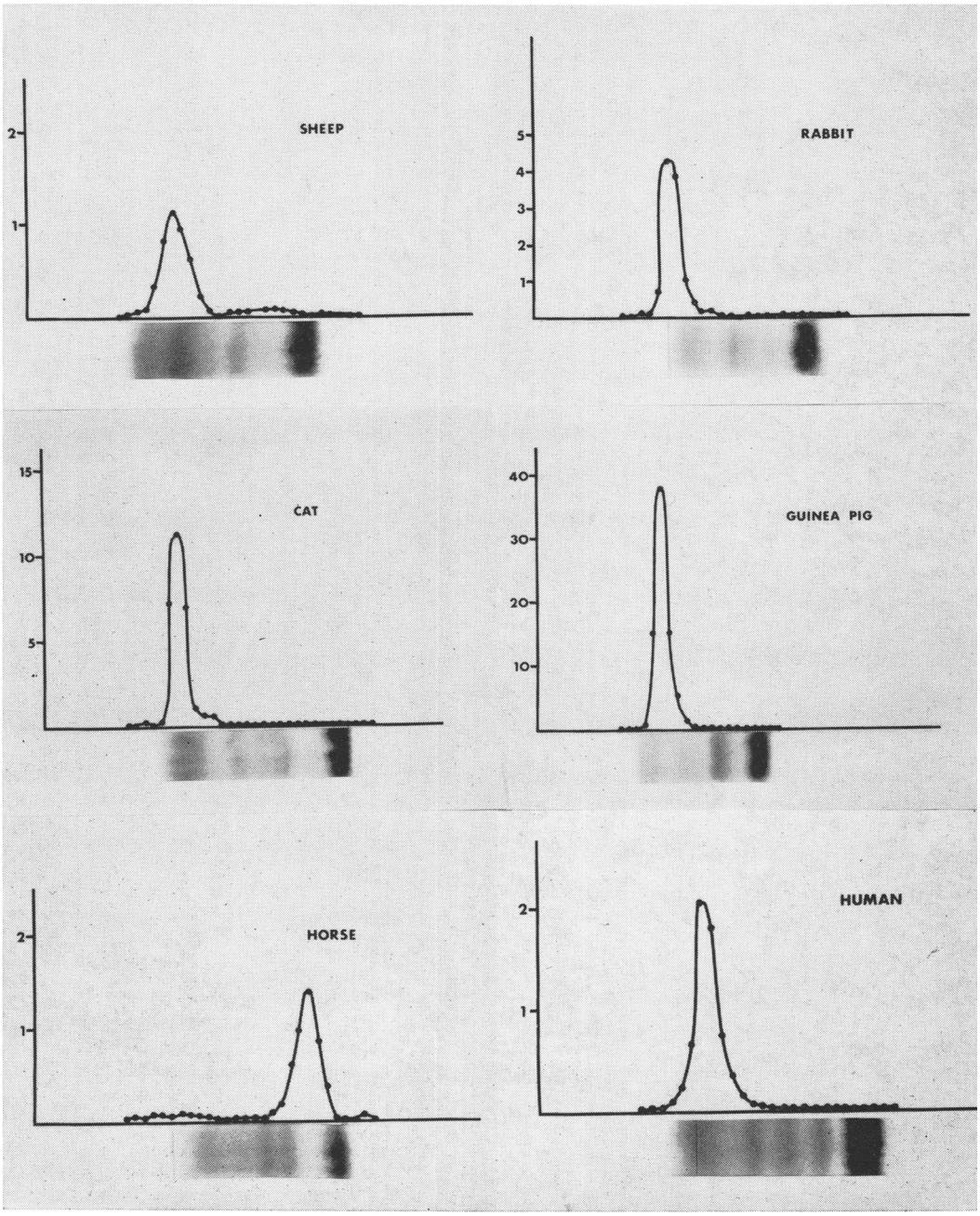

FIG. 1. Electrophoretic patterns of serum amylase and protein in species exhibiting single peaks of saccharogenic activity. The latter is expressed on the ordinates in terms of Somogyi units.

scure and knowledge concerning its origins is embryonic. The total amylase activity in the systemic circulation of the normal animal would be expected to reflect the ubiquity of distribution of this enzyme in various body tissues. To the extent that these tissues vary in their amylase content, the serum amylase activities would likewise vary. Thus, the extremely high levels of amylase activity in rat, guinea pig and pig sera prompts the conjecture that some tissues of these animals are rich in amylase and that the polymorphic

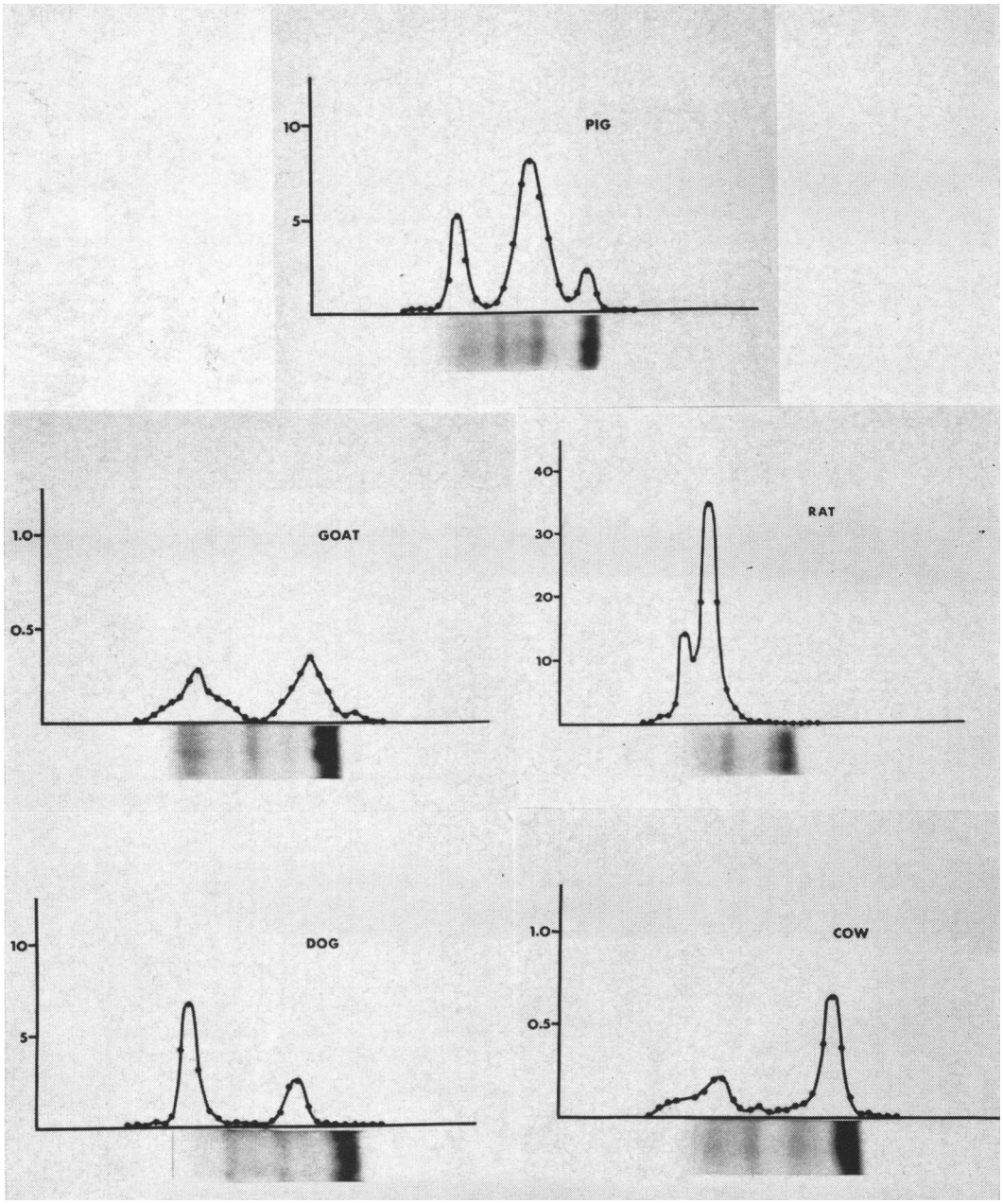

FIG. 2. Electrophoretic patterns of serum amylase and protein in species exhibiting multiple peaks of saccharogenic activity. The latter is expressed on the ordinates in terms of Somogyi units.

electrophoretic pattern in the rat and pig further denotes this. The electrophoretic monomorphism exhibited by amylase in guinea pig serum does not necessarily indicate a single derivation site. It may be, rather, that the amylases from the tissues in this animal do not differ sufficiently in charge to make separation possible on paper.

It would be of interest to find, if possible, an explanation for the variable electrophoretic behavior of amylase in the serum of certain mammals. That such differences are not simply the result of binding by serum proteins is suggested by the fact that in some species amylase is demonstrable in electrophoretic areas devoid of stainable quantities of protein. Using a special buffer system, it also has been shown that amylase can be clearly separated electrophoretically from the γ-globulin fraction in human serum(8).

Summary. The electrophoretic distribution of amylase has been examined in sera collected from 11 mammalian species including man. Monomorphic patterns were noted in human, sheep, rabbit, guinea pig, cat and horse serum, whereas amylase behaved polymorphically in specimens from the rat, pig, cow, dog and goat. These findings suggest that, in the latter species, amylase circulates in molecular forms that are distinguishable by electrophoresis on paper. The possible relationships between the paper electrophoretic behavior of serum amylase and tissue derivations are discussed.

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Absence of Serum Alpha-2 Globulin in Niemann-Pick Disease. A Measure of Hepato-Cellular Involvement.* (31386)

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Hepatomegaly is one of the major clinical manifestations of Niemann-Pick disease, a sphingomyelin-cholesterol lipidosis(1). Crocker and Farber performed liver function tests on 10 patients with Niemann-Pick disease. They found a poor correlation between involvement of the organ, and the various flocculation tests, bromsulfalein excretion, and serum protein concentration and distribution (2). They concluded that "there is considerable need for laboratory procedures that would give a more profound evaluation of the undoubted hepatic malfunction present in this disease." Although serum alpha-2 globulin is frequently decreased in primary liver disease when measured by paper electrophoresis with a veronal buffer(3), Crocker and Farber found that the electrophoretic pattern in Niemann-Pick disease was normal. Since Tris borate buffer fractionates the alpha globulins into 3 distinct bands, compared to only 2 obtained with veronal buffer, the sera of 10 patients with Niemann-Pick disease were analyzed by paper electrophoresis with the Tris borate buffer. It was observed that alpha-2 globulin (the second of the 3 alpha bands) was absent in all 10 patients. A similar pattern was obtained from 13 out of 14 sera of patients with primary or secondary

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