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AUTOLYSIS OF FETAL PIG TISSUES

by

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Certain experimental studies have indicated that the enzymes involved in autolysis may be reversible in nature and thus play a part in the synthesis of protein, given favorable conditions. Should this be true, one might expect to find a higher degree of activity of these enzymes in the rapidly growing tissues of the fetus than in the more "stationary" adult tissues. The presence of proteolytic enzymes in the fetal tissues of a variety of animal species has been demonstrated (1-5) but in the comparison of autolytic rates at various stages of fetal development with the adult there has been considerable disagreement. Undoubtedly, certain of the discrepancies among the earlier publications arose from a lack of the more modern methods now available. Mendel and Leavenworth (2) observed that in an acid medium fetal pig liver autolysed as well as does the adult liver and that there were no significant changes in activity with variations in the fetal age. But this work was done before methods for the accurate estimation of H ion concentration had been developed and they simply added known amounts of acid; it is now well known that small variations in pH have a marked influence on enzymatic activity. Moreover, the quantities of acid which they used would be insufficient, theoretically, to attain the optimal pH for catheptic action.

and this is in agreement with their observation, that, with both fetal and adult liver, only approximately one-third of the protein present was digested. In a series of well-controlled experiments, using glycerol extracts of the chick embryo and gelatin, ovalbumen or lecithovitellin as substrate, Mystkowski (5) has concluded that "the activity of cathepsin does not undergo much change during the whole developmental period of the chick embryo." The actual proteolysis of the chick embryo tissues was not reported.

A study of the autolysis of mammalian fetal tissues at various accurately controlled H ion concentrations seemed to us to be indicated in order to see if, with fetal development, differences in enzymatic activity or protein fragility could be detected. The liver and muscle of pig fetuses were used.

Litters were obtained at the slaughter-house directly after killing and the weight and crown-rump length of each member determined. From the averages of these values it was possible to estimate the approximate intrauterine age of each litter (6). In the liver autolysis studies the ages ranged from 30 to 112 days although in no one experiment was the age variation of the litters greater than 9 days. The average gestation period of the sow is 114 days (6); the pig fetus at 30 days is about one inch in length

and to obtain the livers from smaller forms seemed impractical. For muscle autolysis studies two intra-uterine age groups, 94 to 103 days and 67 to 71 days, were used, muscle tissue being obtained from the limbs and from along the backs of the fetuses.

The tissue (liver or muscle), dissected from the fetuses, was ground finely in a meat chopper. 200 g. of the ground material were treated with 50 cc. of toluol, the mixture made up to a final volume of 1000 cc. and "homogenized" in a Waring mixer. 50 cc. portions of this were placed in small flasks and usually adjusted to pH values of 2, 3, 4, 5, 6 and 7.5. In a second series, 3 cc. of 20% hemoglobin (7) were added to each aliquot and the pH values then adjusted as above. After initial samples were taken, the flasks were kept at 37°C., further sampling being performed at the end of the first, third, fifth and tenth days. The H ion concentrations were determined at frequent intervals as long as shifting occurred and the necessary adjustments made. The samples, removed at the appointed times, were treated with trichloracetic acid (final conc. of 5%). To determine the extent of digestion the total nitrogen and tyrosine contents of the filtrates were determined, using the macro-Kjeldahl and Folin-Ciocalteu methods respectively. (The total nitrogen of the filtrates will be referred to as soluble

nitrogen).

The moisture content of each batch of tissue was determined by weighing out samples, immediately after grinding, and drying them at 100°C. to constant weight. The results obtained are listed in Table I.

The values presented in Figure 1 were obtained from the autolysis of fetal livers (72 to 79 days) and have been chosen as typical of all the intrauterine age groups studied. There is a striking similarity to the autolysis of adult pig liver; the maximal values in the neighborhood of pH 4 are suggestive of catheptic proteolysis. Most of the digestion has taken place by the end of the third day although there are still small increases at the end of the tenth day when the experiment was terminated. The quantities of soluble nitrogen and of tyrosine are closely parallel.

Digestion of the added substrate (hemoglobin) occurred in all age groups and the values contained in Table II are typical.

In Figure 2, the percentages of nitrogen rendered soluble during autolysis have been plotted against the periods of digestion(pH 4). Should there be a difference in enzymatic activity at some stage of fetal development, one would expect to see evidence of this at the end of the first day when proteolysis is still rapidly progressing. At this time the percentages of

solubilized nitrogen vary from 62.4% (30 to 39 days) to 46.5% (53 to 60 days), the other values lying within this range in no definite order of magnitude with respect to intrauterine age. (Adult liver lies about midway - 55.6%). At the end of ten days the greatest amount of digestion had occurred with adult liver (84.4%), the percentages of solubilized nitrogen for the fetal livers varying from 79.7% (94 to 103 days) to 72.9% (110 to 112 days). A similar treatment of the results obtained in the series with added substrate also gives no evidence of any progressive variation in the proteolytic enzyme content of the liver during intrauterine development.

Fetal pig muscle, like the adult tissue, autolysed slowly and to a much lesser extent than does the liver. Maximal digestion occurred in the neighborhood of pH 4 and a comparison of the results obtained at this degree of acidity using fetal and adult tissue, with and without hemoglobin, is presented in Figure 3. Unfortunately, at this H ion concentration, there is marked isoelectric precipitation which made sampling difficult and probably accounts for certain irregularities in the values. Differences in the extent of digestion at the end of the first day are probably not significant. The extent of autolysis at the end of the tenth day is not much greater in fetal than in adult

muscle although with the 67 to 71 day group there is some increase; but with the addition of hemoglobin the differences are marked and probably lie beyond the range of experimental error. In the consideration of these results, however, it should be pointed out that the protein content per unit volume of brei was much greater when adult muscle was used (5.3 mg. of coagulable nitrogen per cc.) than in the experiments with fetal muscle (1.74 and 1.64 mg. of coagulable nitrogen per cc. respectively), a variation in keeping with the difference in moisture contents of the two types of tissue (Table I). The actual quantities of nitrogen solubilized over the ten day period are considerably greater for adult than for fetal muscle (Figure 4), a finding which might be expected in view of the greater protein content of the adult muscle breis. But in the parallel series where hemoglobin had been added, the ten day values for fetal and adult muscle are identical in spite of the fact that there is more available substrate in the case of the adult tissue.

Conclusions

The results of this investigation indicate that the autolysis of fetal pig liver proceeds at roughly the same rate as does that of the adult tissue. This would suggest that the enzyme concentrations of the two types of tissue are fairly similar although a less concentrated or a less active enzyme acting upon a more friable substrate might conceivably give similar results. However, the parallel results observed in the two types of tissue with the addition of hemoglobin tends to disprove the latter view. The extent of autolysis at the end of the tenth day was always slightly greater with adult tissue which probably means that a greater proportion of this tissue is available for catheptic action. The addition of hemoglobin resulted in an increase in the amount of soluble nitrogen with both fetal and adult livers but on the basis of the ratio $\frac{\text{soluble N}}{\text{total N}}$ the extent of digestion was with one exception slightly less than when liver was used alone. (Table III)

Cathepsin-like activity is also present in fetal muscle and this tissue, like adult muscle, seems to be relatively poor in available substrate. When the additional substrate was added, the proportion of digestion at the end of ten days was greater with fetal than with adult muscle yet the actual amounts

of protein digested were the same in both, the differences in extent of digestion lying in the greater initial protein content of the adult muscle brei. This would indicate that the enzymic activity of fetal muscle does not markedly differ from that of adult tissue.

Table I

Moisture Contents of Hog Liver and Muscle

Fetal and Adult

Fetal liver - 110-112 days - - - - - -	80.7%
Fetal liver - 94-103 days - - - - - -	82.8%
Fetal liver - 72-79 days - - - - - -	82.2%
Fetal liver - 53-60 days - - - - - -	81.8%
Fetal liver - 44-48 days - - - - - -	81.1%
Fetal Liver - 30-39 days - - - - - -	81.8%
Adult liver - - - - - - - - - - - - -	70.9%
Fetal muscle - 94-103 days - - - - - -	88.0%
Fetal muscle - 67-71 days - - - - - -	90.8%
Adult muscle - - - - - - - - - - - - -	73.8%

Table II

Autolysis of fetal pig liver - with and without hemoglobin

Soluble nitrogen in mg. per cc. of filtrate

pH		Initial	1st day	3rd day	5th day	10th day
		mg/cc	mg/cc	mg/cc	mg/cc	mg/cc
2	Liver	.20	.22	.22	.22	.25
2	Liver & Hb	.14	.17	.17	.17	.20
3	Liver	.20	.62	.67	.67	.73
3	Liver & Hb	.22	.84	.95	.92	.95
4	Liver	.22	.67	.90	.92	.95
4	Liver & Hb	.20	.81	1.06	1.09	1.15
5.	Liver	.20	.56	.76	.78	.87
5	Liver & Hb	.17	.62	.78	.84	.95
6	Liver	.17	.36	.45	.48	.56
6	Liver & Hb	.17	.34	.53	.59	.70
7.5	Liver	.17	.22	.28	.25	.28
7.5	Liver & Hb	.17	.22	.22	.25	.31

Table III

Percentage of protein digested, tenth day, pH 4

Tissue	Percentage digestion	Percentage digestion with added hemoglobin
Adult liver	84.4	82.1
Fetal Liver--110 to 112 days	72.9	73.8
" " -- 94 to 103 days	79.7	70.4
" " -- 72 to 79 days	77.6	73.7
" " -- 53 to 60 days	75.4	68.1
" " -- 44 to 48 days	74.3	70.5
" " -- 30 to 39 days	79.0	77.8
Adult muscle (1)	14.3	21.7
Adult muscle (2)	17.4	21.5
Fetal muscle--94 to 103 days	20.7	43.8
" " --67 to 71 days	29.3	45.1

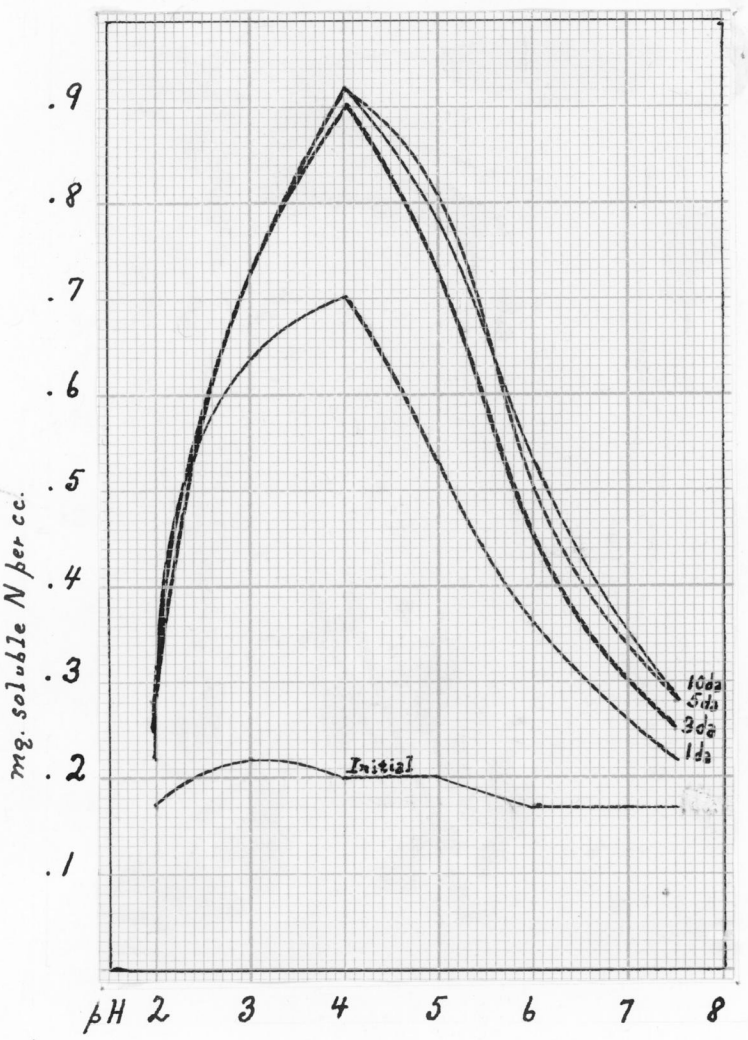

Figure 1a The Autolysis of Fetal Pig Liver

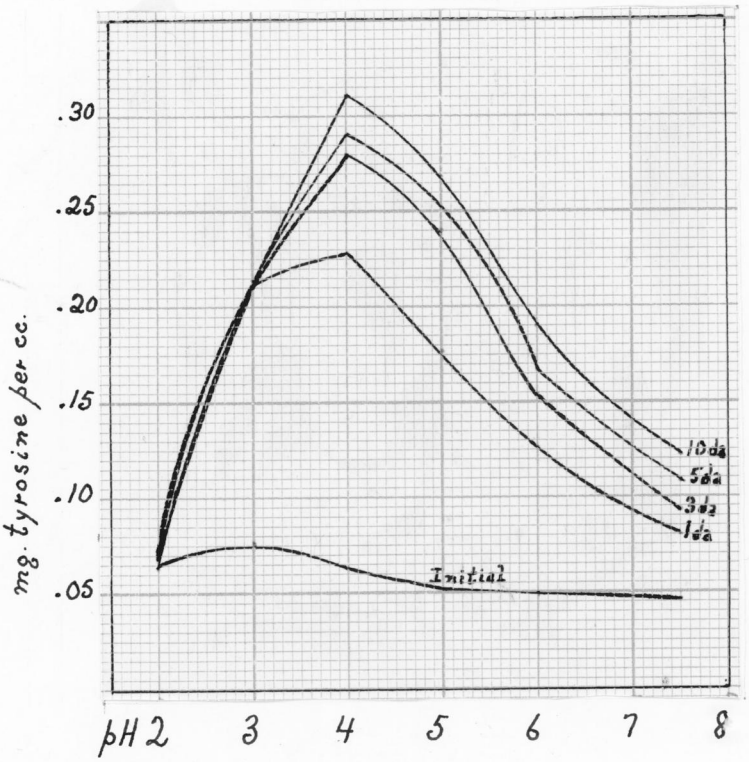

Figure 1b The Autolysis of Fetal Pig Liver

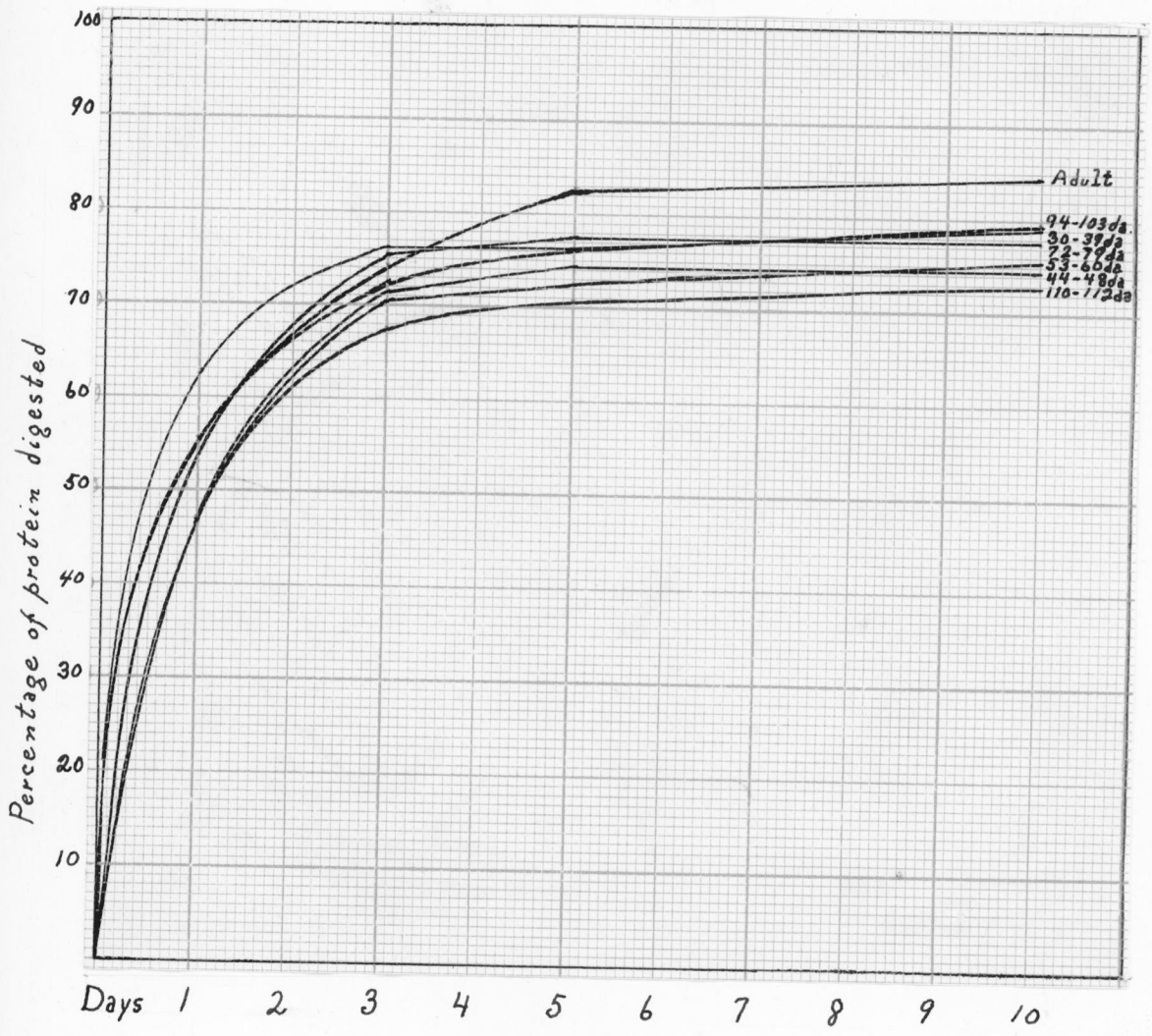

Figure 2 The Autolysis of Pig Liver - Various Intrauterine Ages Compared with Adult - pH 4

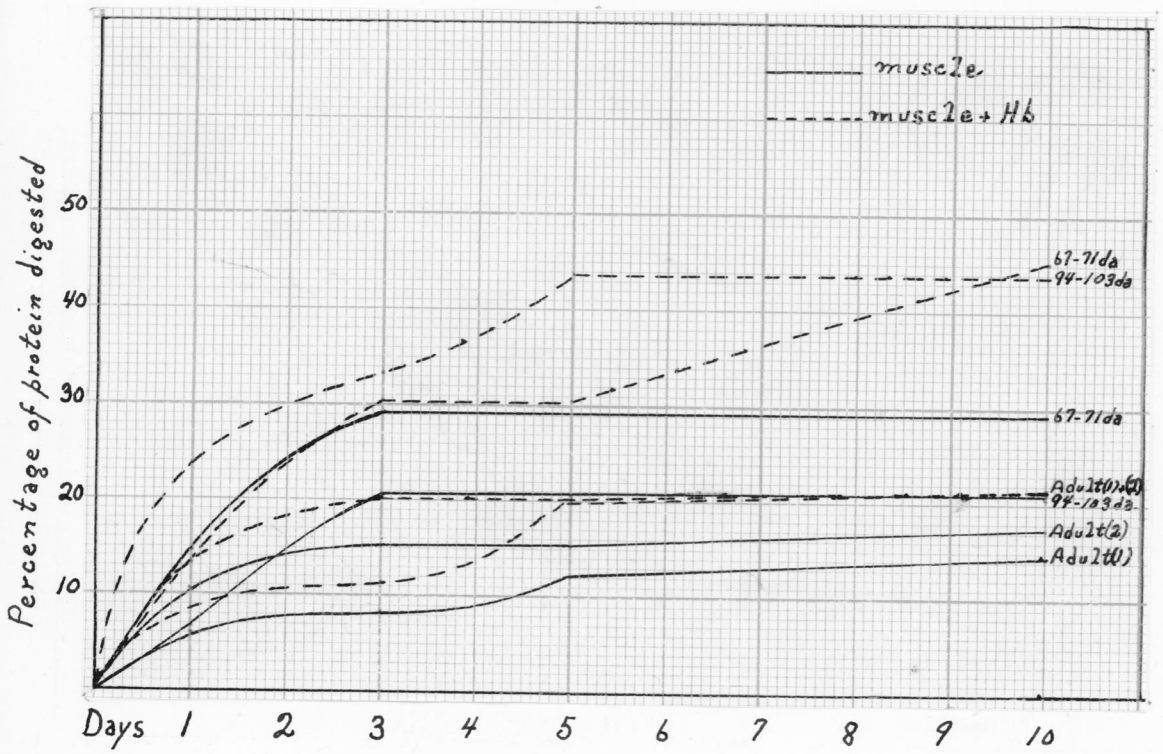

Figure 3 The Autolysis of Pig Muscle -

Fetal and Adult - pH 4

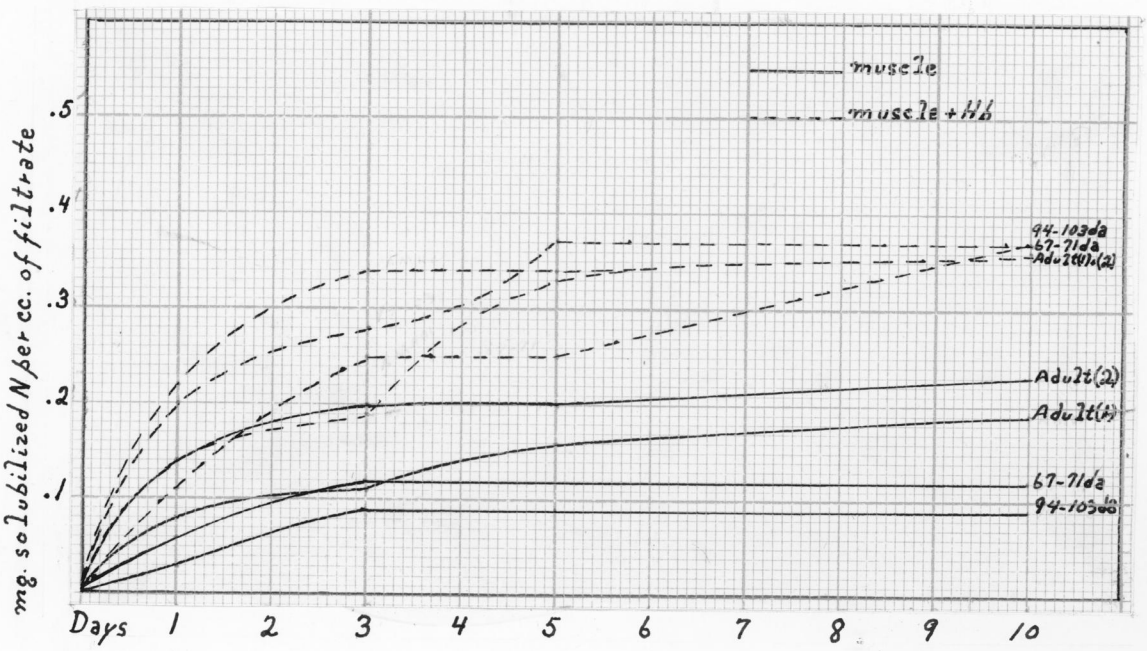

Figure 4 The Autolysis of Fetal and Adult Muscle - pH 4

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APPROVED BY

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DATE

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