# THE CONDITIONS OF CONVERSION OF PANCREATIC ZYMOGENS INTO ENZYMES.

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(*From the Physiological Laboratory, Oxford.*)

CONTENTS.



OUR knowledge concerning the zymogen of the proteolytic ferment of the pancreas has received scarcely any additions since 1875, when Heidenhain first demonstrated the existence of such a body[1]; and though Hammarsten[2], and also Langley[3], have shown that the rennet ferment of the stomach does not exist as such in the gastric mucous membrane, but as a zymogen, no proof seems to have been adduced of the existence of a similar body in the pancreatic tissue. It seemed of interest, therefore, to make further observations upon these two closely related ferments, and to determine more exactly the conditions under which the zymogens of trypsin and of rennin set free their enzymes, the rapidity of such transformation, and the degree of stability possessed by the enzymes, once they are formed.

[1] *Arch. f. d. ges. Physiol.* x. p. 557. 1875.

[2] *Jahresb. ü. d. Fortschr. d. Thier-Chem. Wiesbaden.* 1872.

[3] This *Journal*, III. p. 287. 1882.

The methods of estimation of the tryptic power of extracts (dependent on fibrin digestion), and of the rennet power (dependent on the metacasein reaction of milk), have already been described in detail[1], and so need not be referred to again. The extracts used were invariably made by treating 1 part of the finely minced or chopped gland substance with 4 parts by volume of the extracting liquid, and so were of approximately 20 % strength. The extracting liquids employed were the following: chloroform water; ·9 % sodium chloride solution containing chloroform; saturated sodium chloride solution; water containing 25 % of alcohol (methylated spirit); and glycerin, either concentrated, or containing 25 % or 50 % of water. Most of these liquids were employed by Roberts[2], but the normal saline and diluted glycerin solutions do not seem to have been used before.

*The Development of Ferment Activity in Kept Extracts.*

A large number of observations were made upon the ferment activity of kept extracts, the extracting liquid being left in contact with the gland substance, and small quantities of it filtered off as required. In the first few series to be described, the rennet ferment alone was estimated; but, as will be shown subsequently, the tryptic ferment as a rule varies qualitatively in a more or less similar manner to the rennetic, though its quantitative relationship is by no means constant. The accompanying figure shows the rennetic values obtained with extracts of an ox pancreas. This gland was obtained half-an-hour after death, and was immediately dissected as free as possible from fat and connective tissue, passed several times through a mincing machine, and mixed with the extracting liquids. During the first few days the extracts showed practically no rennetic power, but between the fifth and ninth days of extraction the R of the alcoholic extract increased from 1·65 to 167: between the 13th and 19th days that of the normal saline extract increased from 7·8 to 203: and between the 19th and 33rd days that of the aqueous and of the saturated saline extracts increased in a similar manner. That is to say, in the course of a few days the rennetic value increased from practically nothing to its maximum, or nearly its maximum, value. As can be seen from the curves in the figure, this maximum was maintained for only a few days'

[1] This *Journal*, xxvi. p. 405, and xxvii. p. 174.
[2] "Lumleian Lectures," 1880.

in the case of the alcoholic and aqueous extracts, and then fell rapidly away. In the normal saline extract it was maintained for about six

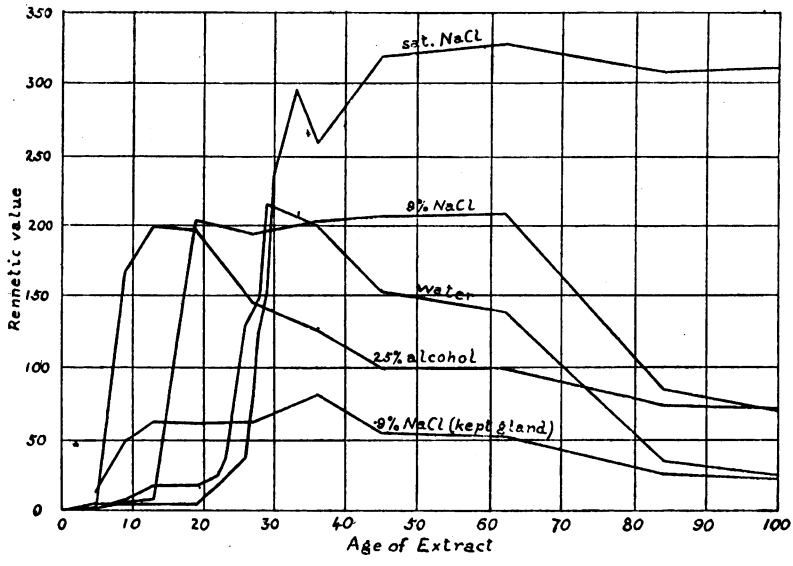


Fig. 1. Ox pancreas.

weeks, whilst in the saturated saline extract it had not diminished at all even three months after the beginning of the observations, and even 13 months after had fallen only to 174.

In the case of the aqueous and saturated brine extracts, the development of the rennetic power was followed more closely, and the following values were obtained:

| Aqueous extract | | Saturated salt extract | |
|---|---|---|---|
| 19 days | 16·8 | 19 days | 3·9 |
| 21 | 21·4 | 22 | 18·2 |
| 22 | 24·3 | 26 | 37·2 |
| 23 | 36·0 | 27 | 77 |
| 26 | 129 | 28 | 127 |
| 28 | 149 | 29 | 153 |
| 29 | 216 | 30 | 238 |
| 36 | 200 | 33 | 295 |

In the aqueous extract, we see that the R increased from 36·0 to 129 between the 23rd and 26th days, and again from 149 to 216 between the 28th and 29th days. In the saline extract, the most rapid rise was between the 26th and 28th days, when the R increased from 37·2 to 127, and between the 29th and 30th, when it increased from 153 to 238.

An explanation of this sudden development of ferment power will be afforded further on, though no exact reason can be given as to why one extract should develop its activity sooner or later than another. It does not in most cases depend on the nature of the extracting liquid, for sometimes one extract will become active first, and sometimes another. Glycerin extracts form an exception, for in them the liberation of the enzyme is always more delayed than in other media. In the case of this ox pancreas, for instance, a concentrated glycerin extract had a rennetic power of only 2·8 after four months. However, on addition of 50 % of water to some of the glycerin and gland substance (after it had undergone 19 days' extraction), the rennetic power increased to 142 in eight days, so that diluted glycerin does not cause much retardation.

The lowest curve in the above figure shows the values obtained with a normal saline extract of some of the gland substance which had been kept 60 hours before extraction. This and other curves of kept gland substance, which are given in subsequent figures, will be referred to later on.

The next figure shows the values obtained with extracts of pig's pancreas. There was practically no liberation of ferment during the first seven days of extraction, and then the rennetic power of the

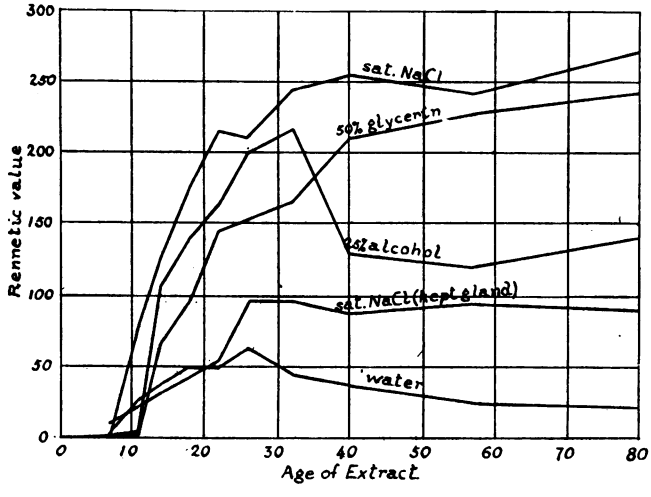


Fig. 2. Pig's pancreas.

saturated saline extract began to rapidly develop. That of the alcoholic extract followed suit four days later, and the glycerin extract, made with

glycerin containing 50 % of water, also began to liberate its ferment, though not so rapidly. Taking them as a whole, these extracts did not attain their maximum activity nearly so rapidly as the ox pancreas extracts. The most rapid development occurred in the alcoholic extract, in which the R increased from 4·9 to 108 between the 11th and 14th days. The aqueous extract began to develop its ferment after the seventh day, but its maximum value was only about a fourth that attained by the other extracts. In fact, chloroform water proved to be the least efficient of all the extracting media employed. As far as the rennetic value is concerned, saturated salt solution was perhaps the most efficient, for in this medium a higher rennetic value is developed even than in glycerin, and the ferment seems to remain equally well preserved. For general purposes, however, glycerin is by far the best medium.

The observations on this pig's pancreas were continued for 130 days, but the values at the end of this period differed very little from those found after 80 days, so they are not introduced in the figure.

In the next figure are recorded the values obtained with extracts of sheep's pancreas. The glands of two sheep were obtained, and were

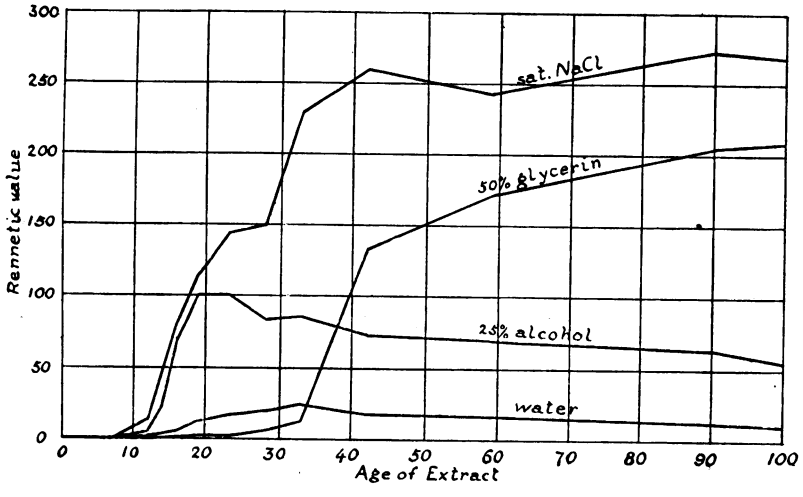


Fig. 3. Sheep's pancreas.

extracted within two hours of death of the animals. None of the extracts showed any activity after seven days' extraction, and very little even after twelve. The saturated salt extract had then begun to become active, and within the next four days its R increased from 14·2

to 80·0. Still neither this nor any of the other extracts developed their activity as rapidly as the pig and ox pancreas extracts. The R of the alcoholic extract increased from 6·0 to 69·6 between the 12th and 16th days, but the maximum value, reached after 19 days, was less than half that attained by the brine and 50% glycerin extracts. This latter extract developed its activity somewhat quickly, its R increasing from 12·2 to 133 between the 33rd and 42nd days. The aqueous extract was even less efficient than in the case of pig's pancreas, the maximum value attained by it not being a tenth of that reached by the brine extract.

In all the observations on the development of ferment activity the extracts were kept at room temperature. If kept at 38°, the ferment is liberated much more rapidly, but it is even more rapidly destroyed. In the accompanying table are recorded a few observations made on portions of the alcoholic extracts of sheep and pig's pancreas above mentioned, and here it will be seen that at 38° the extract of sheep's pancreas reached its maximum activity in 3 days, instead of the 13 days required at room temperature. The maximum was not a sixth as great, however. Some of the extract filtered off from its gland substance

| Extract | Originally | 1 day | 2 days | 3 days | 4 days | 6 days | 8 days | 10 days | 13 days |
|---|---|---|---|---|---|---|---|---|---|
| Sheep (kept with gland substance at 16°) | ·5 | — | — | — | 6·0 | 25·7 | 69·6 | — | 100 |
| Sheep (kept with gland substance at 38°) | ·5 | — | 9·1 | 15·4 | 7·9 | 5·7 | 6·3 | 1·6 | |
| Sheep (filtered and kept at 38°) | ·5 | 2·8 | 10·0 | 10·0 | 7·2 | 5·1 | 5·7 | — | 3·6 |
| Pig (kept with gland substance at 16°) | ·9 | — | 1·8 | — | — | 4·9 | — | — | 139 |
| Pig (filtered and kept at 38°) | ·9 | 1·4 | 53·5 | 82·5 | 54·5 | 30·7 | — | 21·1 | — |

reached a smaller maximum after 2 days. Filtered extract of pig's pancreas reached a maximum after 3 days, instead of the 27 days required at room temperature. This maximum was fairly high, it being more than a third of the room temperature value. Still it is clear that observations on extracts kept at 38° instead of at the room temperature teach one much less than they do as to the processes involved.

The next figure gives the values for dog's pancreas extracts. The gland was obtained from a large bull-dog, immediately after death, the animal being killed two or three hours after a meal. The extracts were remarkable for the rapidity with which they increased and decreased in ferment power. After two days' contact with the gland

substance they had practically no rennetic power, but six days later, when they were next examined, they had attained to nearly their

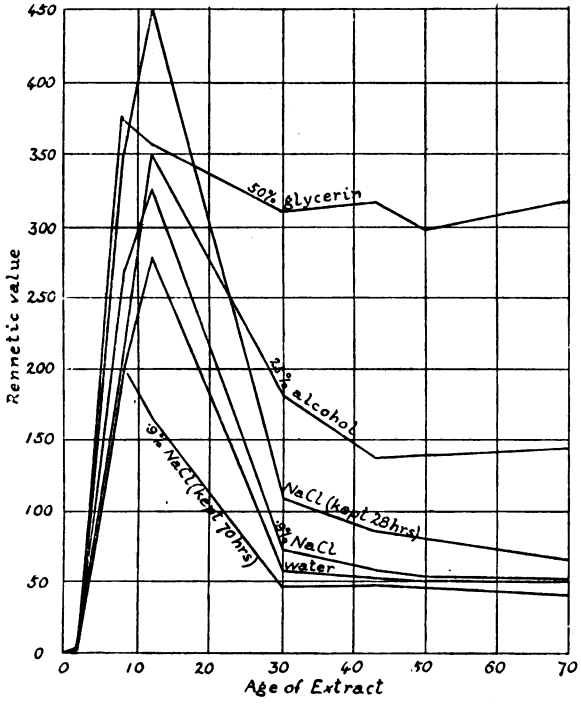


Fig. 4. Dog's pancreas.

maximum strength. The aqueous extract had increased in rennetic value from 2·6 to 200; the normal saline from 3·2 to 267; the alcoholic from 3·6 to 212; the 50% glycerin from 2·9 to 372; and the saline extract of gland substance kept 28 hours before extracting, from 1·7 to 347. The fall in rennetic power was almost as rapid as the rise, for between the 12th and 30th days the aqueous extract fell from 278 to 58, the saline from 326 to 72, the alcoholic from 349 to 180, and the extract of 28 hours' kept pancreas from 450 to 110. Subsequent to this, however, the fall in power was very slow. The glycerin extract, as usual, retained its activity, though there was a slight falling off even in its case.

It might perhaps be thought that these striking variations were brought about by rapid alterations in the temperature of the atmosphere. This could scarcely have been the case, however, as they were

begun in the middle of October. The observations on ox, pig and sheep's pancreas were begun during August.

In the next three series of observations to be described, the tryptic value of the extracts was determined as well as the rennetic. The accompanying figure shows the T and R values of the alcoholic and normal saline extracts of sheep's pancreas, the values obtained for the glycerin extracts being omitted so as to avoid confusion. The T values

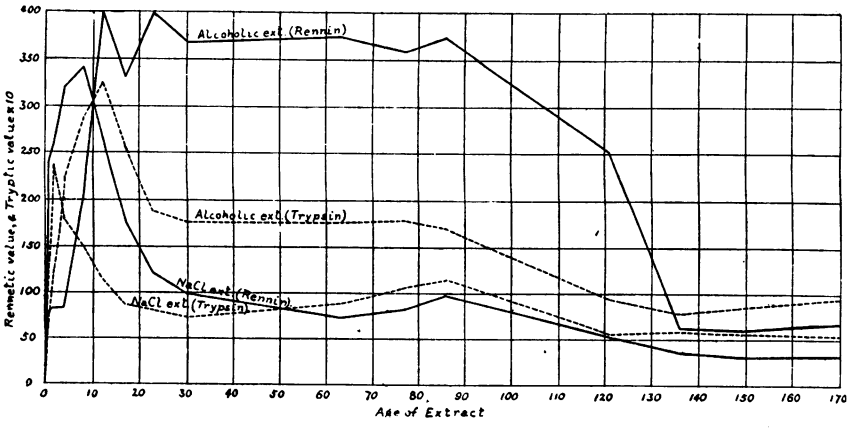


Fig. 5. Sheep's pancreas.

are indicated by dotted line curves. From these curves, and also from the table of values given below, one can see that the ferments were liberated with much greater rapidity than in the case of the extracts hitherto mentioned. A similar rapid liberation of enzyme was observed in the other series of observations to be described. The probable explanation of this lies in the fact that in all these cases several glands were used, and minced together, whilst in the observations just described only a single gland was used, except in those on the sheep, when there were two. Thus in the present instance four sheep's glands were minced up, and in the extracts of pig's pancreas described below, respectively four and two glands. Presumably the slight differences in the nature of the gland tissue obtained from different animals leads to a more rapid liberation of ferment.

To return to the discussion of the figure, we see that as regards the alcoholic extract, both the tryptic and rennetic ferments began to develop at once, and both reached their maximum after 12 days. After this the tryptic power rapidly fell away, but the rennetic remained nearly at its maximum for 74 days before it began to

decline. Subsequently, however, it fell off very rapidly indeed. In the table is given the ratio between each pair of R and T values, and it will be seen at a glance that this is highly variable. Between the 1st and 4th days of extraction it dropped from 12·9 to 3·7, and then 19 days later it rose to over 20. At this level it remained for 98 days, and then suddenly dropped again. The great variations of the ratio during the first few days of extraction are due to the fact that during this time the rennetic power of the extract remained almost constant, whilst the tryptic power rapidly increased. Such a striking difference in the times of liberation of these enzymes from their zymogens is quite exceptional, it not being observed in any other instance. In fact, one may state in most definite terms that when an extract liberates the tryptic enzyme, it liberates also the rennetic. Roughly speaking, one may also say that as a rule, when an extract begins to deteriorate in respect of the tryptic enzyme, it sooner or later deteriorates in respect of the rennetic. Hence it follows that the curves of rennetic values given above for ox, sheep, pig, and dog's pancreatic extracts afford a fair indication as to the times and manner of liberation of the tryptic ferment, and to some extent also suggest the times at which this ferment underwent deterioration. It should always be remembered, however, that the relationship between the two ferments is only roughly qualitative. Quantitatively, the resemblance is but slight.

As regards the normal saline extract, it will be seen in the figure that the correspondence between the values of the two ferments is more close, especially after the first thirty days. Still the tryptic ferment reached its maximum after 41 hours' extraction, and the rennetic only after 8 days. From these respective periods onwards both ferments diminished rapidly in activity. From the 63rd to the 86th day, however, both underwent a slight secondary rise, and then gradually diminished again. The ratio of R to T thus remained moderately constant from the 63rd day onwards. With one exception, the ratio also remained fairly constant from the 1st to the 17th day, it then being about three times as great as that observed subsequently.

In the case of the 75 % glycerin extract, the ratio between R and T remained even more constant, for between the 23rd and 149th days it varied only from 3·3 to 5·8. One cannot attach much importance to the values observed during the first few days, as they were so small that they could not be determined with much exactness. Both

| Extracting liquid (sheep's pancreas) | | 17 hrs. | 41 hrs. | 4 days | 8 days | 12 days | 17 days | 23 days | 30 days | 63 days | 77 days | 86 days | 121 days | 136 days | 149 days | 176 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 % Alcohol | T | 5·8 | 12·3 | 22·4 | 28·7 | 32·6 | 25·7 | 18·8 | 17·6 | 17·6 | 17·8 | 17·0 | 9·4 | 7·9 | 8·5 | 9·9 |
| | R | 75 | 82 | 83 | 207 | 399 | 331 | 399 | 368 | 374 | 359 | 373 | 252 | 62 | 61 | 69 |
| | R÷T | 12·9 | 6·7 | 3·7 | 7·2 | 12·2 | 12·9 | 21·2 | 20·9 | 21·2 | 20·2 | 21·9 | 26·7 | 7·8 | 7·1 | 7·0 |
| | % destroyed | — | *72·8* | — | *67·4* | — | — | *59·8* | *67·3* | *53·2* | *45·4* | *46·7* | *21·5* | *18·1* | *8·2* | *10·9* |
| ·9 % NaCl | T | 12·1 | 23·7 | 17·8 | 15·0 | 11·6 | 8·7 | 8·0 | 7·3 | 9·0 | 10·7 | 11·4 | 5·6 | 5·9 | 5·8 | 5·6 |
| | R | 239 | 256 | 319 | 341 | 267 | 176 | 122 | 100 | 73 | 83 | 99 | 56 | 37·3 | 32·0 | 33·8 |
| | R÷T | 19·8 | 10·8 | 17·9 | 22·7 | 23·1 | 20·1 | 15·2 | 13·7 | 8·1 | 7·7 | 8·7 | 10·0 | 6·3 | 5·5 | 6·0 |
| | % destroyed | — | *76·9* | — | *51·7* | — | *44·8* | | *42·4* | — | *38·4* | *27·1* | *18·6* | *31·4* | *8·0* | *20·5* |
| 75 % Glycerin | T | 0·0 | ·3 | ·9 | 1·6 | 1·7 | 3·2 | 5·4 | 12·2 | 72·2 | 69·1 | 69·1 | 56·1 | 53·9 | 61·2 | 47·9 |
| | R | 1·7 | 3·0 | — | 18·5 | 14·4 | 21·8 | 25·9 | 55·0 | 238 | 275 | 346 | 326 | 293 | 291 | 308 |
| | R÷T | — | 10·0 | — | 12·0 | 8·6 | 6·9 | 4·8 | 4·5 | 3·3 | 4·0 | 5·0 | 5·8 | 5·4 | 4·8 | 6·4 |
| | % destroyed | — | — | — | — | — | — | — | *20·8* | *77·0* | *73·2* | *83·7* | *80·4* | *80·9* | *82·7* | *82·2* |
| 75 % Glycerin (gland kept 63 hrs.) | T | — | — | 9·0 | 12·0 | 11·9 | 9·3 | — | 10·3 | 15·3 | 13·6 | 18·2 | 9·1 | 8·6 | 13·5 | 10·9 |
| | R | — | — | — | 367 | 372 | 331 | — | 411 | 420 | 377 | 413 | 423 | 399 | 358 | 396 |
| | R÷T | — | — | — | 30·6 | 31·3 | 35·6 | — | 39·9 | 27·5 | 27·7 | 22·7 | 46·5 | 46·6 | 26·5 | 36·4 |
| | % destroyed | — | — | — | *56·5* | — | — | — | *57·7* | *34·2* | *39·0* | *40·5* | *32·1* | *46·0* | *36·3* | *41·2* |

ferments began to develop very gradually for the first 30 days, and then increased more rapidly, so that the tryptic ferment reached its maximum after 63 days' extraction, and the rennetic ferment after 86 days.

The glycerin extract of gland substance kept 63 hours before extraction will be mentioned again further on, but attention may here be drawn to the remarkably high ratios obtained over the whole series of observations. They varied from 22·7 to 46·6, or were on an average more than five times as great as those observed with the glycerin extract of normal gland substance.

Comparing the various extracts together, we see that the glycerin extract attained the highest tryptic value, it being more than twice as active as the alcoholic extract, three times as active as the normal saline extract, and four times as active as the glycerin extract of kept gland substance. The rennetic values show much less variation, the maxima of the different extracts varying only from 341 to 423. Curiously enough the absolute maximum was attained by the extract of kept gland substance, which maintained a uniformly high rennetic value throughout.

In the next figure are given the tryptic and rennetic values

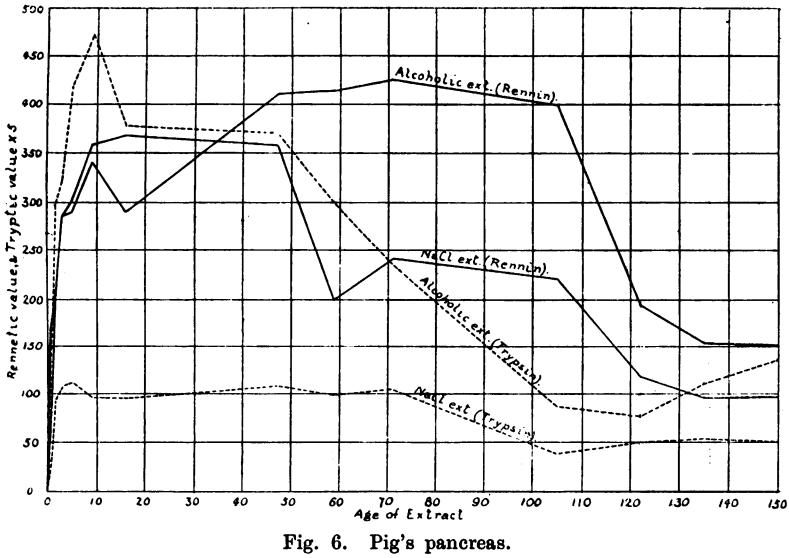


Fig. 6. Pig's pancreas.

obtained with alcoholic and normal saline extracts of pig's pancreas. The alcoholic extract attained its maximum tryptic value after 9 days,

| Extracting liquid (pig's pancreas) | | 16 hrs. | 40 hrs. | 3 days | 5 days | 9 days | 16 days | 47 days | 59 days | 71 days | 105 days | 122 days | 135 days | 158 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 % Alcohol ... ... | T | 30·1 | 59·3 | 64·6 | 83·7 | 94·6 | 75·6 | 74·3 | 60·2 | 47·2 | 17·6 | 15·5 | 22·6 | 29·9 |
| | R | 167 | 207 | 285 | 290 | 340 | 290 | 411 | 414 | 425 | 400 | 194 | 155 | 151 |
| | R÷T | 5·6 | 3·5 | 4·4 | 3·5 | 3·6 | 3·8 | 5·5 | 8·5 | 9·0 | 22·7 | 12·5 | 6·9 | 5·1 |
| | % destroyed | *55·0* | *63·5* | — | *59·4* | — | *57·5* | *53·8* | *43·9* | *32·1* | *10·1* | *3·5* | *10·5* | *16·0* |
| ·9 % NaCl ... ... | T | 5·6 | 18·5 | 21·6 | 22·3 | 19·2 | 19·1 | 21·9 | 19·8 | 21·2 | 7·9 | 10·2 | 11·0 | 10·6 |
| | R | 97 | 210 | 285 | 301 | 358 | 368 | 358 | 200 | 241 | 221 | 119 | 97 | 99 |
| | R÷T | 17·4 | 11·4 | 13·2 | 13·5 | 18·6 | 19·3 | 16·3 | 10·1 | 11·4 | 27·9 | 11·7 | 8·8 | 9·3 |
| | % destroyed | *+43·4* | *+5·1* | — | — | *31·2* | *42·1* | *20·3* | *25·1* | *37·4* | *27·3* | *13·6* | *32·2* | *10·7* |
| 75 % Glycerin ... ... | T | — | 1·2 | — | 1·8 | 1·9 | 6·0 | 206 | 207 | 208 | 159 | 156 | 143 | 139 |
| | R | — | 9·6 | — | 11·3 | 24·8 | 43·0 | 442 | 424 | 501 | 512 | 460 | 484 | 472 |
| | R÷T | — | 7·9 | — | 6·4 | 13·1 | 7·1 | 2·1 | 2·0 | 2·4 | 3·2 | 3·0 | 3·4 | 3·4 |
| | % destroyed | — | — | — | — | — | *+67·1* | *61·7* | *68·2* | *70·9* | *67·2* | *71·5* | *69·0* | *73·8* |
| 75 % Glycerin ... ... (gland kept 25 hrs.) | T | — | 6·1 | — | 10·1 | 11·7 | 11·9 | 15·9 | 26·6 | 26·4 | 16·5 | 21·8 | 35·0 | 32·9 |
| | R | — | 109 | — | 108 | 128 | 129 | 219 | 235 | 271 | 359 | 349 | 272 | 301 |
| | R÷T | — | 18·0 | — | 10·7 | 11·0 | 10·8 | 13·8 | 8·8 | 10·3 | 21·8 | 16·0 | 7·8 | 9·1 |
| | % destroyed | — | — | — | — | — | *+2·7* | *+112* | *+37·6* | *1·1* | *4·4* | *12·4* | *8·4* | *35·2* |

and then fell away somewhat, but it was not until after 47 days' extraction that it began to deteriorate rapidly. The rennetic value did not reach its maximum till after 47 to 71 days' extraction, and did not begin to fall away rapidly till after 105 days. By this time the tryptic ferment had dwindled down to less than a fifth of its former value, so that the ratio of R to T underwent considerable variations. During the first 47 days' extraction, however, it remained nearly constant, it varying only from 3·5 to 5·6. It then gradually increased to a maximum of 22·7, and then fell away again.

The normal saline extract was somewhat remarkable, in that its tryptic value reached a very low maximum after 5 days' extraction, and then remained practically unchanged for 66 more days. Its rennetic value, on the other hand, was not much inferior to that observed with the alcoholic extract. It practically reached its maximum after 9 days' extraction, remained constant for 38 days, and then somewhat rapidly deteriorated. The ratio of R to T did not undergo any very marked variations for the first 71 days, but then it suddenly rose to its maximum of 27·9, and as suddenly fell.

The 75 % glycerin extract behaved very much like the similar extract of sheep's pancreas. The tryptic power increased only very slowly at first, and then more or less suddenly rose to a maximum. This it maintained for only 24 days, and then underwent a considerable deterioration. The glycerin extract of sheep's pancreas was noticed to undergo a lesser deterioration after remaining 23 days at its maximum. The explanation of this falling off almost certainly lies in the auto-digestion which these extracts underwent. During the first few days after the maximum activity had been attained, the extract could be filtered off fairly readily from the gland substance, but after this with ever-increasing difficulty. Finally the gland substance and glycerin became mingled into an almost homogeneous pulp.

The rennetic value of this glycerin extract developed at the same time as the tryptic, though it did not reach its absolute maximum till some weeks later. Also it did not undergo the subsequent diminution noticed in the case of the tryptic ferment. Still from the 47th day onwards the ratio remained fairly constant, it varying only from 2·0 to 3·4.

Comparing the various extracts together, we see that the maximum tryptic value of 208, attained by the glycerin extract, was more than double that of the alcoholic extract, and nearly ten times that of the

normal saline extract. It was, in fact, the most active preparation I obtained. Some idea of its activity is furnished by comparing it with other preparations. Kühne's dried pancreas preparation, digested with 5 parts of ·1 % salicylic acid for 20 hours at 38°, was found to give an extract with a tryptic value of 3·94, or was about a fiftieth as active. A soluble trypsin powder (Grübler) was found in 10 % solution to have a tryptic value of 23·2. Now ·1 gm. of this preparation was said to be capable of dissolving 8 gm. of fibrin, hence 1 c.c. of the glycerin extract of pig's pancreas should have been capable of dissolving 72 gm. of fibrin.

As in the case of the sheep's pancreas extracts, the rennetic values differed much less than the tryptic, the maxima attained by the various extracts varying only from 359 to 501. The rennetic ferment is evidently more stable than the tryptic, and is much less affected by the nature of the extracting liquid. Taking the extracts as a whole, one may conclude that the rennet ferment is liberated somewhat more slowly than the tryptic, and so does not attain its maximum quite so soon. Similarly it does not begin to deteriorate so quickly as the tryptic ferment, but once it does begin it may fall off very rapidly indeed.

In the accompanying table are given the means of all the ratios of R to T obtained with each extract. Except in the case of the

| | Sheep | Pig |
|---|---|---|
| Alcohol ... ... | 14·0 | 7·3 |
| ·9 % NaCl ... ... | 13·0 | 14·6 |
| Glycerin ... ... | 6·3 | 4·9 |
| Glycerin (kept gland) | 33·7 | 12·6 |

normal saline extract, all the ratios are higher for sheep's pancreas than for pig's. We may probably conclude, therefore, that sheep's pancreas contains a relatively greater amount of rennet ferment than of tryptic; or, otherwise stated, the pig's pancreas contains a relatively greater amount of tryptic ferment. The high average ratio yielded by saline extract of pig's pancreas is merely the result of the tryptic ferment failing to develop anything like its full strength.

The rennetic and tryptic powers of a number of other extracts were also compared, the tryptic power being estimated by another but less accurate method of fibrin digestion. Taking the mean ratio of R to T in all the observations as unity, then the mean ratio for five different extracts of human pancreas was found to be ·45; for

four of pig's pancreas, ·61; for five of dog's pancreas, ·97; for four of sheep's pancreas, 1·17, and for six of ox pancreas, 1·82. Apparently therefore, the human pancreas contained about four times more trypsin, relative to its rennin, than did ox pancreas, whilst pig's pancreas contained about three times as much, and dog's pancreas about twice as much.

Another short series of observations was made upon extracts of pig's pancreas, only two glands being used in this case. Nevertheless, as can be seen from the figures given in the subjoined table, the alcoholic extract began to develop its tryptic and rennetic power immediately. So much so, indeed, that the tryptic power reached

| Extracting liquid (pig's pancreas) | | 1 day | 2 days | 4 days | 11 days | 21 days | 41 days |
|---|---|---|---|---|---|---|---|
| 25 % Alcohol ... ... | T | 10·2 | 50·4 | 48·6 | 49·2 | 52·6 | 26·9 |
| | R | 48·7 | 150 | 203 | 239 | 238 | 245 |
| | R÷T | 4·8 | 2·3 | 4·2 | 4·9 | 4·5 | 9·1 |
| | % dest. | *38·4* | *52·2* | *42·2* | *42·8* | *55·2* | *21·9* |
| 75 % Glycerin ... ... | T | — | 3·0 | 3·8 | 7·0 | 8·4 | 13·1 |
| (gland kept 20 hrs.) | R | — | 37·8 | 51 | 56 | 79 | 96 |
| | R÷T | — | 12·6 | 13·5 | 8·0 | 9·4 | 7·3 |
| | % dest. | — | — | *+59·0* | — | *+51·6* | *+14·5* |

nearly its maximum after two days. The actual maximum, attained after 21 days, was not much more than half that obtained in the previous observations on pig's glands. The rennetic power was likewise considerably less. The ratio of R to T remained fairly constant for the first 21 days, and was less than half as great as that of the 75 % glycerin extract of kept gland substance. In fact, the ratios obtained for each of these extracts bear a close resemblance to those obtained with the similar extracts of the former glands.

### *The change in stability of the trypsin in kept extracts.*

In a former paper (*loc. cit.* p. 414) it was shown that the trypsin of active extracts was an exceedingly unstable body, so much so that if the diluted extract were kept at 38° with ·4 % sodium carbonate, about 65 % of it was destroyed in an hour. On the other hand, comparatively inactive extracts, with about a hundredth the tryptic power, had only about 7 % of their ferment destroyed per hour by similar treatment, and extracts of intermediate degrees of activity showed intermediate degrees of ferment destruction. These results

were taken to indicate that the ferment trypsin is not a single chemical substance, but that there exist a series of trypsins of gradually increasing degrees of stability. When an extract is kept, therefore, and gradually undergoes deterioration, the most sensitive trypsins would be first destroyed, and the least sensitive ones last.

These conclusions are fully supported by the results since obtained. In almost all the above recorded determinations of the tryptic value of extracts of sheep and pig's pancreas, the rate of destruction of the ferment was determined in addition. This was done by diluting a known quantity of an extract to ten times its volume with ·4% $Na_2CO_3$, keeping it for exactly an hour at 38°, and then determining its tryptic value in the ordinary way. Such a quantity of extract was taken as to give about the same digestion time as the untreated extract. Supposing, for instance, an extract with a T of about 40 were expected to have 50% of its ferment destroyed, then ·5 c.c. of the extract would be treated with $Na_2CO_3$ at 38°, and would then be found to give a fibrin digestion time of 30 minutes, or the same time that ·25 c.c. of the untreated extract would afford.

The percentages of ferment destroyed per hour are given in the tables of tryptic and rennetic values obtained with extracts of sheep and pig's pancreas. As regards the alcoholic extract of sheep's pancreas, we see that during the first 8 days of extraction, about 70% per hour of the ferment was destroyed. Then, as the tryptic power diminished, the stability of the ferment increased, and between the 23rd and 86th days, when the tryptic value averaged 17·8, the destruction rate averaged only 54·5%. The considerable drop in tryptic power between the 86th and 121st days was accompanied by a corresponding increase of stability, so that from the 121st day onwards, when the tryptic value averaged 8·9, the destruction rate averaged only 12·2%, or less than a quarter as much as in the previous period.

The normal saline extract of sheep's pancreas did not afford such striking evidence as the alcoholic, in that the tryptic value was irregular. This fell from its maximum of 23·7 on the 2nd day to 7·3 on the 30th day, and then rose again to 11·4 on the 86th. During all these 86 days it must therefore have contained considerable quantities of unconverted zymogen. However, we see that when the extract was most active, it was also most unstable, and when least active, least unstable. Thus from the 121st day onwards, when the tryptic value averaged 5·7, the destruction rate averaged 19·6%, or about half as

much as between the 17th and 86th days, when the tryptic value averaged 9·5, and the destruction rate 38·2 %.

The ferment of the glycerin extract, once it had developed its full power, proved to be most extraordinarily unstable; more so, in fact, than in any other extract obtained. Also the instability did not seem to diminish, in spite of the tryptic value falling off by a third, and from the 63rd day onwards, when the tryptic value averaged 61·4, the destruction rate averaged 80·0 %. The destruction rates after 63 and 77 days' extraction were in fact somewhat smaller than those observed subsequently, but this may very well have been due to the presence of small quantities of unconverted zymogen, from which the enzyme was liberated on exposure to the $Na_2CO_3$ at 38°. Thus the extract had a destruction rate of only 20·8 % after 30 days' extraction, and before the tryptic power had much developed. Also, as we shall see later, extracts may even contain such large quantities of zymogen as to exhibit a considerable increase of tryptic power when kept at 38°.

The glycerin extract of kept gland substance seemed somewhat more unstable during the first 30 days than it did later on, although the tryptic power had slightly increased. Still the difference observed is within the limits of experimental error. This is necessarily somewhat large, depending as it does on the determination of two ferment values, either or both of which may be—exceptionally—as much as 10 % in error, though probably the average error is not more than 5 %. The tryptic value of this extract was very variable from day to day, probably owing to the presence of unconverted zymogens, but on the whole it may be considered to have kept roughly constant. Taking a mean of all the observations, therefore, we find that the tryptic value averaged 11·8, and the destruction rate 42·6 %.

The results obtained with the alcoholic extract of pig's pancreas bear a close resemblance to those obtained with the similar extract of sheep's pancreas. On the whole, however, the ferment seemed distinctly more stable, in spite of the fact that the extract was about three times as active. Thus the maximum destruction rate was only 63·5 %, whilst with sheep's pancreas values of 72·8, 67·4 and 67·3 % were obtained. The stability of the trypsin increased regularly as the activity of the extract decreased. Thus both tryptic value and destruction rate experienced correspondingly slight falls between the 47th and 59th days, and 59th and 71st days, and correspondingly big ones between the 71st and 105th days. The T in this latter case fell

from 47·2 to 17·6, and the destruction rate from 32·1 % to 10·1 %. The minimum T was reached on the 122nd day, and from this time onwards it increased so considerably that by the 158th day it had almost doubled itself. Large quantities of unconverted zymogen must still have been present therefore.

The normal saline extract gave somewhat variable results, but between the 9th and 71st days, when the tryptic value remained nearly constant, the destruction rate likewise remained nearly constant. The former then averaged 20·2, and the latter 31·2 %. Between the 71st and 105th days the tryptic value diminished by more than half, and from that time onwards increased again slightly, it averaging 10·6. The destruction rate also fell off, but not so much as one would expect, it now averaging 18·8 %. The increase in the tryptic value between the 105th and 135th days shows that, as in the alcoholic extract, unconverted zymogen must still have been present. During the first two days of extraction this was so much the case that the extract showed a distinct increase in tryptic power on keeping with $Na_2CO_3$ at 38°, so the liberation of fresh enzyme must have been considerably more than sufficient to counterbalance the destruction. In the glycerin extracts this liberation of enzyme was even more marked. Thus the tryptic power of the 16 days' extract of fresh gland was found to be increased by 67 %, and in another determination, in which the time of exposure to $Na_2CO_3$ at 38° was only 8 minutes, an increase of 24·3 % was observed. The glycerin extract of gland substance kept 25 hours before extraction showed an increase of 112 % after 47 days' extraction, whilst even after 135 days the destruction rate was only 8·4 %, although the tryptic value was 35·0. Hence even then a good deal of unconverted zymogen must have been present.

The glycerin extract of normal gland substance showed a practically constant destruction rate from the 47th day onwards, in spite of the tryptic value falling off from 208 to 139. As the same phenomenon was observed on the corresponding extract of sheep's pancreas, it can scarcely be an accidental result. Presumably a considerable quantity of ferment in these very active extracts, perhaps a third or even a half of it, consists of trypsin molecules of the same degree of instability, so that only when most or all of this trypsin is destroyed can the more stable trypsins reveal their presence by a diminished destruction rate.

The mean destruction rate for this extract of pig's pancreas is 68·9 %, as compared with the 80·0 % value observed with sheep's pancreas. We see therefore that the extracts of pig's pancreas, though

about three times more active than the corresponding extracts of sheep's pancreas, are distinctly more stable than they. One may accordingly conclude that the trypsins of one series of extracts do really differ from those of the other series as regards stability.

The nature of the extracting liquid seems to be of comparatively little direct importance in the question of the stability of the trypsins, this depending chiefly or entirely on the tryptic value of the extract. In the accompanying figure are plotted out all the decomposition rates obtained with the various extracts of sheep's pancreas, even those recorded in the above-mentioned paper being introduced. Though

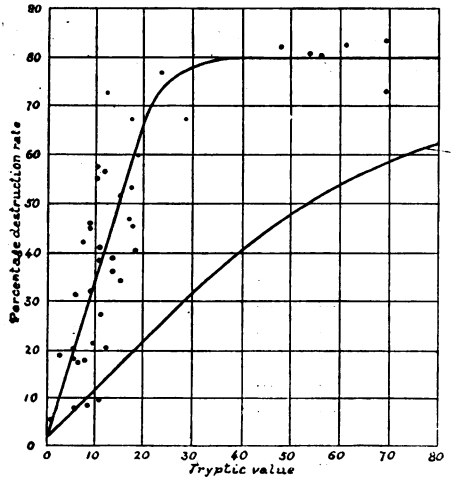


Fig. 7.

somewhat variable, they are on the whole in fair agreement with the mean curve which has been drawn through them. This curve therefore indicates the average destruction rate of extracts of sheep's pancreas of all degrees of tryptic value. The curve has been drawn to indicate that at least 2 % per hour of the ferment in an extract would undergo destruction, however feeble it be, and also that all extracts with a tryptic value of 40 and upwards possess the same destruction rate of 80 % per hour. For the sake of clearness, the destruction rates obtained with extracts of pig's pancreas have not been introduced in this figure, but a part of the mean curve itself is given. This is founded on the results obtained with all the extracts of pig's pancreas above mentioned (except those of kept gland substance), on the second series of alcoholic extract values, and on the few values recorded in the paper aforesaid.

The entire difference in the form of the curve from that obtained with sheep's glands well indicates that extracts of glands from different animals, though of the same tryptic value, may differ very widely in the stability of their ferment.

It is probable that the destruction rates observed for trypsins hold approximately also for rennins. It was found[1] that active extracts, with an average R of 395, if kept at 38° with 10 times their volume of water for an hour, had on an average 49·3 % of their ferment destroyed. Less active extracts, with an average R of 228, had 39·4 % destroyed: those with an R of 66·6, 24·3 %, and those with an R of 27·4, 11·6 %. No further observations were made upon the destruction rates of rennins, but some were made on trypsins, after the extracts had been kept for an hour at 38° with water, instead of ·4 % $Na_2CO_3$. These observations are collected in the accompanying table, where the percentages of the destruction rates by water on those by $Na_2CO_3$ are also given. Though the values are distinctly irregular, we see that the

| Extract | T | % ferment destroyed by water | Relation of water destruction rate to that of $Na_2CO_3$ |
|---|---|---|---|
| Glycerin ext. pig ... ... | 139 | 31·6 | 42·8 |
| Glycerin ,, sheep ... ... | 61·2 | 53·0 | 64·1 |
| Alcohol ,, pig ... ... | 29·9 | 15·2 | 95·0 |
| NaCl ,, pig ... ... | 19·5 | 25·3 | 75·3 |
| Alcohol ,, sheep ... ... | 17·8 | 36·0 | 79·3 |
| NaCl ,, pig ... ... | 10·6 | 7·4 | 69·2 |
| Glycerin ,, sheep (kept gland) | 10·5 | 31·6 | 55·9 |
| NaCl ,, sheep ... ... | 5·8 | 6·2 | 77·5 |

water in all cases destroyed less than the $Na_2CO_3$, the water destruction rate being on an average 69·9 % on the $Na_2CO_3$ destruction rate. Now if the destruction rates for trypsin obtained in the observations on sheep and pig's pancreases be reduced to 70 % their value, numbers are obtained which are closely comparable to the rennin destruction rates above mentioned. In still another respect, therefore, does the rennet ferment resemble the tryptic.

### *The action of enzymes on zymogens.*

We have seen that pancreatic extracts frequently remain comparatively inactive for a variable number of days, and then more or less rapidly develop their rennetic and proteolytic power. What is it that

[1] This *Journal*, XXVII. p. 195.

initiates and carries on this conversion of the zymogen? The observations to be described seem to show that the enzyme itself is the exciting agent, and that once a small quantity of "active" enzyme is formed, it quickly brings about the conversion of some of the zymogen, the enzyme thus set free bringing about the conversion of more zymogen, and so on, till all or nearly all of the zymogen is converted into enzyme.

We saw that the 75 % glycerin extracts of sheep and pig's pancreas developed their ferment activity comparatively slowly. Portions of the extracts were filtered off from their gland substance and kept separate, and were then found to increase in tryptic power more slowly than ever. If, however, water were added, the paralysing influence of the glycerin was removed, and the ferment was liberated with a rapidity which increased with the dilution. Thus from the values given in

| | 1 day | 2 days | 4 days | 8 days | 13 days |
|---|---|---|---|---|---|
| 75 % glycerin ext. pig (undiluted) | 1·8 | — | 1·9 | — | — |
| + ½ vol. water | 2·3 | 2·7 | 5·1 | 7·8 | 87·1 |
| 1 vol. „ | — | 4·2 | 7·0 | 75·8 | — |
| 2 vols. „ | 3·9 | 6·3 | 59·0 | 71·8 | — |

the accompanying table, we see that on the addition of half a volume of water the extract increased its tryptic value to 7·8 in 8 days. On addition of 1 volume of water, nearly the same value was reached in 4 days, and on the addition of 2 volumes of water, in a little over 2 days. Once the extracts had reached a tryptic value of about 7, the ferment began to be liberated very rapidly, and the T of the extract containing half a volume of water rose to 87·1 in the next five days; of that containing 1 volume of water, to 75·8 in 4 days, and of that containing 2 volumes, to 59·0 in 2 days.

In the next table are given the values obtained with the glycerin extract of sheep's pancreas. This had spontaneously developed a T of 3·2, so on dilution the rise of tryptic power was more rapid.

| | Tryptic values after | | | Rennetic values after | |
|---|---|---|---|---|---|
| | 1 day | 2 days | 4 days | 1 day | 2 days |
| 75 % Glycerin ext. sheep (T=3·2) (R=21·8) | — | 4·9 | 5·4 | — | 44·3 |
| + ½ vol. water | 9·6 | 18·6 | 30·5 | 95 | 225 |
| + 1 vol. „ | 12·3 | 33·9 | — | 185 | 267 |

The extract diluted with 1 volume of water attained as great a T in two days as the extract diluted with half a volume attained in four.

The rennetic values were also determined, and, as can be seen in the table, were found to increase more or less *pari passu* with the tryptic values.

That this conversion of zymogen into enzyme is brought about by the action of the enzyme itself is proved by the fact that if small quantities of an active extract be added, the rate of conversion of the zymogen is enormously increased. In the accompanying table are shown the results obtained by the addition of respectively 1, 3, and 10 % of an active glycerin extract of pig's pancreas (which had a T of 141), to another 75% glycerin extract of pig's pancreas diluted with 2 volumes of water. The T of this extract at the beginning was only

| Active extract added | Tryptic value induced in inactive extract after | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 hr. | 1 hr. | 2 hrs. | 5½ hrs. | 9 hrs. | 24 hrs. | 2 days |
| 1 % | 8·4 | — | — | 20·9 | 32·9 | 66·6 | 88·1 |
| 3 | 13·7 | — | 28·4 | 34·2 | 51·5 | 89·1 | 95·6 |
| 10 | 16·7 | 26·4 | — | 46·3 | 97·5 | 111·1 | 102·6 |

·85, and, owing to the very small quantity of ferment it contained, it increased only to 1·14 in four days, when similarly diluted. Practically the whole of the rapid liberation of enzyme shown by the values in the table was brought about, therefore, by the added extract. The actual tryptic value of the added extract has been subtracted from these values, but it is necessary to make another very considerable correction before the true rate of enzyme production can be estimated. Thus it was found that the active glycerin extract was able to act upon the glycerin extract of zymogen even after this had been mixed with fibrin and ·4 % $Na_2CO_3$ (for the purpose of determining its tryptic value), and liberate from it a considerable amount of enzyme. The amount of enzyme so liberated is indicated in the second column of the table, under the heading "0 hour." We see that when 1 % of active extract was added to 1 c.c. of glycerin extract of zymogen mixed with fibrin and $Na_2CO_3$, it liberated sufficient enzyme to give a T of 8·4 (the T of the extract added, viz., 1·41, being of course subtracted from the T actually found). Similarly the addition of 3 % of active extract gave a (corrected) T of 13·7. and of 10 %, one of 16·7. To estimate approximately the actual amount of ferment liberated apart from what occurs during digestion, one must therefore subtract these amounts from the corresponding values given in the table. We then find that the addition of 10 % of active extract in 1 hour at room temperature liberated ferment giving a T of $26·4 - 16·7 = 9·7$. If one subtracts the same

amount, viz., 16·7, from the tryptic values obtained after the active extract had acted for 5½ or more hours, however, one falls into greater and greater error. Thus it is obvious that when the active extract has already liberated half or more of the enzyme from its zymogen, it will have a correspondingly smaller amount of zymogen left to act upon during the fibrin digestion, and so the true correction one ought to apply is much less than at first.

An accurate estimation of the rate of liberation of the ferment is thus almost hopeless, but the values given in the table may be taken to show that the addition of 1 % of active extract takes about 6 hours to liberate ferment giving a T of 13; the addition of 3 %, about 2 hours to liberate the same amount of ferment; and of 10 %, about 1½ hours. Or again, as regards the nearly complete conversion of the zymogen into the enzyme, we see that 1 % of active extract effected about as much change in 2 days as 3 % did in 1 day, and as 10 % did in about 8 hours.

If the extract be kept at 38° instead of at room temperature (about 17°), the conversion of zymogen into enzyme is very much more rapid, but the enzyme liberated is so rapidly destroyed that the results obtained are not very striking. The accompanying table shows the values obtained with some of this same glycerin extract (diluted with two volumes of water). These observations were made only a day earlier than the ones at 17°, so the same corrections will apply.

| Active extract added | Tryptic value induced in inactive extract after | | | |
|---|---|---|---|---|
| | 0 min. | 10 mins. | 1 hr. | 2 hrs. |
| 1 % | 8·4 | 9·4 | 12·8 | 20·7 |
| 3 | 13·7 | 22·8 | 29·7 | — |

Here we see that even in 10 minutes quite a considerable amount of enzyme was liberated, for with 3 % of active extract the T was raised from 13·7 to 22·8. During the next 50 minutes, however, the increase was relatively very much less, it being only from 22·8 to 29·7. This must have been chiefly due to the destructive action of the high temperature on the liberated ferment, whereby, after a time, the ferment gets destroyed almost as fast as it is liberated. In any case the rate of liberation of the enzyme never under any circumstances approached that observed by Langley and Edkins[1] in the case of pepsinogen. Thus it was found by them that at 20° C. nearly all the pepsinogen in an aqueous extract of cat's gastric mucous

[1] This *Journal*, VII. p. 403. 1886.

membrane was converted into pepsin in 60 seconds by ·1 % of hydrochloric acid.

Another series of observations at 38° had been made upon this same (diluted) glycerin extract five days before, with the following results.

| Active extract added | Tryptic value induced in inactive extract after | | | | |
|---|---|---|---|---|---|
| | 1 hr. | 2 hrs. | 3 hrs. | 5 hrs. | 9 hrs. |
| nil | 1·1 | 1·4 | 2·5 | 3·2 | 2·7 |
| 1 % | 10·7 | 13·0 | — | 16·4 | 24·2 |
| 3 | 14·9 | 21·5 | — | 27·0 | 32·0 |
| 5 | 21·9 | — | 28·4 | — | — |
| 10 | 25·2 | 31·5 | 36·8 | 34·4 | 36·2 |

Here we see that exposure of the diluted extract to 38° for even nine hours was able to effect very little liberation of enzyme, unless some active extract was added. In fact, the T obtained after nine hours' exposure was less than that obtained after five hours, so the ferment was then being destroyed faster than it was being formed. When 1 % of active extract was added, we see that after respectively one and two hours' treatment tryptic values of only 10·7 and 13·0 were obtained, instead of the values of 12·8 and 20·7 recorded under similar conditions in the above table. Also with 3 % of active extract the T after one hour was only 14·9, instead of 29·7. That is to say, the enzyme was being liberated very much more slowly, so that the addition of 3 % of active extract did not effect much more than did the addition of 1 % five days later. It is true that the extract itself had initially a T of only ·65, instead of ·85, but this slight difference in the amount of ferment present is almost negligible, when we consider that 1 % of active extract means a T of 1·41, and 3 %, a T of 4·23. It seems, therefore, as if the zymogen itself were much less susceptible than it became five days later, or that during this time it underwent some kind of decomposition changes which, though not actually leading to its conversion into enzyme, yet brought it nearer towards the enzymic condition.

Some of the same extract was examined on still two other occasions, with results confirmatory of the view of increased susceptibility to ferment action. For convenience, all the comparative results obtained are collected in the accompanying table. Here we see that twelve days after the first time of examination, the addition of 1 % of active extract induced in one hour a T of 19·2, or about as much as was induced in two hours in the five days' extract. Similarly it was found that in the 25 days' extract, 1 % of active extract induced in 10 minutes

a T 61 % greater than that induced in a similar time in the five days' extract. However, the extract had spontaneously undergone a distinct

| | | After keeping extract for | | |
|---|---|---|---|---|
| | At first | 5 days | 12 days | 25 days |
| Extract (undiluted) ... ... ... ... | ·67 | ·85 | 2·2 | 3·6 |
| +2 vols. water 1 hour at 38° ... ... | 1·1 | — | 3·3 | 4·7 |
| +1 % active ext. 10 mins. | — | 9·4 | — | 15·1 |
| +1 „ „ 1 hour | 10·7 | 12·8 | 19·2 | 21·4 |
| +1 „ „ 2 hours | 13·0 | 20·7 | — | — |
| +3 „ „ 1 hour | 14·9 | 29·7 | 28·0 | — |

rise in tryptic value from the fifth day onwards, but I do not think this was sufficient to account for the increased susceptibility observed. Thus it will be noticed that when kept at 38° for an hour by itself, the diluted extract increased very little in tryptic power. In fact, as we shall see later, the comparatively stable enzyme which must then have been present had very much less "liberating" power than the smaller quantity of highly unstable enzyme which was artificially added. As already mentioned, 1 % of this active extract would of itself mean a T of 1·41, whereas between the 5th and 25th days the extract had spontaneously developed a T of 3·59 − ·85 = 2·64.

On the whole, therefore, I think that we may regard this view of the gradually increasing susceptibility of the zymogens as a highly probable one; though by reason of the numerous sources of error which encompass one on every side, it cannot be accepted as conclusively proven. A somewhat similar hypothesis concerning zymogens was suggested nearly twenty years ago by Langley[1]. To quote his words: "Brücke pointed out that prolonged extraction with water does not take out all the pepsin from the gland cells; the remaining tissue when treated with dilute hydrochloric acid still gives a pepsin-containing extract.... We could scarcely imagine that this could be the case if the zymogen existed in the cells in one state only....The protoplasm of the cells does not at one swoop form zymogen as it occurs immediately previous to its conversion into pepsin, but forms certain intermediate bodies in which the zymogen radicles became more and more isolated. Since the zymogen contains the radicle of the ferment, the ferment will be obtained with greater difficulty from the imperfectly elaborated than from the perfectly elaborated zymogen, *i.e.*, as we ascend from the final mesostate to protoplasm, the ferment will be split off less and less readily," etc.

[1] This *Journal*, III. p. 290. 1882.

I have obtained some evidence which seems to show that these zymogens differ in solubility, the imperfectly elaborated ones being insoluble, and the fully elaborated ones perfectly soluble. Thus some of the alcoholic and normal saline extracts of pig's pancreas already referred to were filtered off from their gland substance after 16 hours' extraction, and kept separate. Next day, both the separately kept extracts and extracts freshly filtered from the gland substance had increased their tryptic value by equal amounts, this T being now doubled in the case of the alcoholic extract, and more than trebled in

| | | 1 day | 2 days | 4 days |
|---|---|---|---|---|
| Alcoholic ext. pig (T=30·1) | Fresh filtrate ... ... | 59·3 | 64·6 | 83·7 |
| | Separately kept filtrate | 61·5 | 47·1 | — |
| NaCl ext. pig (T=5·6) ... | Fresh filtrate ... ... | 18·5 | 21·6 | 22·3 |
| | Separately kept filtrate | 17·9 | 15·1 | — |

the case of the saline extract. The day after that, however, the separately kept extracts had considerably deteriorated in tryptic power, whereas the extracts kept with their gland substance had increased. Presumably in their case the enzyme was undergoing destruction as rapidly, or nearly so, as in the separately kept extracts, but this destruction was more than counterbalanced by the liberation of fresh quantities of enzyme from zymogens which, after 16 hours' extraction, and even after 40 hours' extraction, had been insoluble.

What is true for the tryptic ferment is true also for the rennetic. After five days' extraction some of the filtrate from a saturated salt extract of sheep's pancreas was kept apart from the gland substance and was examined from time to time. At first this extract had no ferment power at all, but five days later it had begun to develop. It was now slightly greater in the separately kept extract than in the freshly filtered, but two days later this latter extract took the lead, and gradually drew further and further away. In fact, the separately kept

| Time | Fresh filtrate | Separately kept filtrate |
|---|---|---|
| Originally (5 days' ext.) | nil | nil |
| 5 days later ... ... | 22 | 30 |
| 6 ,, ... .. | 40 | 43 |
| 7 ,, ... ... | 53 | 50 |
| 11 ,, ... ... | 69 | 58 |
| 14 ,, ... ... | 87 | 64 |
| 37 ,, ... ... | 174 | 61 |

extract remained practically constant after 11 days, whilst after 37 days the freshly filtered extract had attained to treble its rennetic power.

The following values, obtained with a normal saline extract of ox pancreas, afford no evidence of the existence of insoluble zymogens, for the separately kept extract (filtered off after seven days' extraction) attained a slightly higher rennetic value than that kept with its gland substance. It is interesting to note that this latter extract developed its ferment power more quickly than the former, and also showed a more rapid deterioration.

| Time | Fresh filtrate | Separately kept filtrate |
|---|---|---|
| Originally (7 days' ext.) | 4·0 | 4·0 |
| 12 days later ... ... | 203 | 15·9 |
| 29 ,, ... ... | 203 | 222 |
| 77 ,, ... ... | 84 | 107 |

*The action of enzymes from different sources on zymogens.*

Thus far we have discussed only the action of active glycerin extract of pig's pancreas upon inactive glycerin extract. The observations to be described show that there is nothing specific about enzymes, but that active extracts of pancreas, whatever the animal from which the gland has been derived, have a similar power of inducing the conversion of the zymogens in any inactive extract, whatever its nature.

In the accompanying table are shown the tryptic values induced in 75 % glycerin extract of pig's pancreas (diluted with half its volume of water), by the addition of 1 % of various other extracts. The tryptic values of these extracts are given, and we see that the power possessed

| | Tryptic value induced after | | | |
|---|---|---|---|---|
| | 1 day | 2 days | 4 days | 8 days |
| Glycerin ext. pig + ½ vol. water ... ... ... | 2·3 | 2·7 | 5·1 | 7·8 |
| + 1 % NaCl ext. pig (T = 22·0) ... | 3·2 | — | 5·1 | 7·8 |
| ,, alcohol ext. sheep (T = 24·5) | 4·3 | 6·8 | 23·6 | 82·8 |
| ,, alcohol ext. pig (T = 59·3) | 18·4 | 31·1 | 65·1 | 80·8 |
| ,, glycerin ext. dog (T = 71·8) | 13·9 | — | 111·5 | — |
| ,, glycerin ext. man (T = 93·2) | 40·4 | — | 119·2 | — |

by them of setting free enzyme in the glycerin extract varies roughly according to their tryptic power. However, the saline extract of pig's pancreas, though possessing a tryptic value of 22·0, had little or no enzyme liberating power, for the tryptic value of the extract with which it was mixed did not increase any more rapidly than that of the extract to which nothing had been added. Even the alcoholic extract of sheep's pancreas did not, during the first two days, liberate ferment

at nearly so proportionately rapid a rate as the other more active extracts. Thus the alcoholic extract of pig, and the glycerin extract of dog, with only two or three times as great a tryptic value, liberated during the first day about seven times as much ferment. Finally the glycerin extract of human pancreas, with about half as large again a T as these latter extracts, liberated two or three times as much ferment as they did. It therefore follows that, proportionately to their activity, active extracts set free the enzyme from the zymogen much more rapidly than the comparatively inactive extracts.

Another series of observations was made with glycerin extract of sheep's pancreas, the rennet ferment being estimated as well as the tryptic. As this extract was spontaneously undergoing a fairly rapid development of ferment power (cf. the values given in the table on p. 289), it was thought best to subtract these respective values from the corresponding tryptic and rennetic values obtained after one and two days with extract to which 1 % of the various active extracts had been added. These reduced values are given in the accompanying table. Here we see that the saline extract of pig's pancreas had no

| 75 % glycerin ext. sheep + ½ vol. water | T of added extract | T induced after 1 day | T induced after 2 days | R of added extract | R induced after 1 day | R induced after 2 days |
|---|---|---|---|---|---|---|
| +1 % NaCl ext. pig ... | 22·0 | 1·4 | (−·9) | 293 | (−20) | 37 |
| ,, alcohol ext. sheep | 24·5 | 4·6 | 1·7 | 365 | 51 | 114 |
| ,, glycerin ext. dog | 71·8 | 5·8 | — | — | — | — |
| ,, alcohol ext. pig | 74·1 | 8·8 | 9·7 | 287 | 171 | 159 |
| ,, glycerin ext. man | 93·2 | 16·5 | — | 377 | 173 | — |

more action on this glycerin extract than it had on the other. This is true for the rennetic ferment as well as for the tryptic, in spite of the fact that the rennetic value of the extract was a high one. Again, the alcoholic extract of sheep's pancreas had a slight amount of action upon the tryptic zymogen, and a somewhat greater one upon the rennetic. The alcoholic extract of pig's pancreas had a fair amount of action upon the tryptic zymogen, and a very marked one upon the rennetic. This latter fact is the more remarkable, as the rennetic power of the added extract was less than that of the saline extract of pig—though this produced no effect at all—and considerably less than that of the alcoholic extract of sheep, though this produced only a small effect. In fact these observations, and others recorded below, seem to show that the rennet ferment is liberated from its zymogen, not by the rennet ferment, but by the tryptic. Thus the alcoholic extract of pig, with a high tryptic power, had a marked liberating action on the

rennet zymogen, whilst the saline extract of pig, with a low tryptic power, had little or none.

Of the other two extracts recorded in the table, we see that the very active extract of human pancreas produced the maximum amount of action on both the tryptic and rennetic zymogens. The glycerin extract of dog, on the other hand, scarcely liberated as much tryptic ferment as one would have expected.

The observations thus far described were carried out at room temperature (about 15° C.). Another more complete series was made at 38°, the 75 % glycerin extract of pig's pancreas used being diluted with two volumes of water, and kept at 38° for an hour with 10 %—not 1 %—of the active extract to be tested. The tryptic value of the added extract was deducted from the actual tryptic value found, it being assumed (on the strength of a few control observations) that 75% of the ferment thus treated for an hour with glycerin extract and water remained undecomposed. The corrected tryptic and rennetic values induced in the glycerin extracts are given in the accompanying table, together with the actual tryptic and rennetic values of the extracts added. In still another column are given the percentage rates of destruction of the active extracts by ·4 % $Na_2CO_3$. To avoid error as much as possible the values given are the averages of the several different determinations made at about the time.

| | T of added extract | % destruction rate of added extract | T induced in glyc. extract | R of added extract | R induced in glyc. extract |
|---|---|---|---|---|---|
| Glycerin ext. pig + 2 vols. $H_2O$ ... ... | | | 1·06 | | |
| +10 % NaCl ext. sheep ... ... | 5·8 | 19·6 | 1·52 | 36 | 25·4 |
| ,, acid glycerin ext. pig ... | 6·7 | 33·9 | 1·67 | 74 | 25·4 |
| ,, NaCl ext. pig ... ... | 10·6 | 18·8 | 1·69 | 108 | 27·3 |
| ,, alcohol ext. sheep ... ... | 8·2 | 12·2 | 2·55 | 59 | 26·4 |
| ,, glyc. ext. sheep (kept 63 hrs.) | 11·0 | 42·6 | 3·47 | 378 | 29·8 |
| ,, ,, pig (kept 25 hrs.) | 28·4 | 18·0 | 3·95 | 310 | 30·5 |
| ,, alcohol ext. pig ... ... | 19·0 | 10·0 | 8·83 | 168 | 76·0 |
| ,, ,, ,, ... ... | 49·2 | 46·7 | 18·6 | 239 | 121 |
| ,, glycerin ext. woman ... | 24·5 | 70·0 | 18·8 | 100 | 131 |
| ,, ,, ,, sheep ... | 60·4 | 80·0 | 21·7 | 292 | 135 |
| ,, ,, ,, pig ... ... | 141 | 68·9 | 25·2 | 472 | 214 |
| 5 % ,, ,, ,, ... ... | 70·5 | — | 21·9 | 236 | 212 |
| 3 % ,, ,, ,, ... ... | 42·3 | — | 14·9 | 142 | 181 |
| 1 % ,, ,, ,, ... ... | 14·1 | — | 10·7 | 47·2 | 142 |

Considering the tryptic values first, we see that, as before, there is a fairly close relationship between the T of the extract added, and the T

induced in the inactive extract. Even the feeblest extracts, with a T of about 6, were able to set free some enzyme, and the saline extract of pig's pancreas also set free a little, though not in an amount corresponding to its actual tryptic value. One must, however, bear in mind that extracts of the same tryptic value do not necessarily contain ferment of the same degree of stability (as tested by the action of $Na_2CO_3$); for we saw that as a rule extracts of sheep's pancreas were more unstable than those of pig's pancreas with treble the tryptic value. That the capacity of extracts to set free enzyme from zymogen is connected with their stability, as well as with their absolute tryptic value, is to some extent indicated by the contents of this table. Thus we see that the stable glycerin extract of kept pig's pancreas had but little more enzyme liberating power than the less stable glycerin extract of kept sheep's pancreas, though it had two or three times the tryptic power. Also we see that the addition of 1 % of the very unstable glycerin extract of pig's pancreas (which had a T of 141, and of which 1 % would therefore be equivalent to 10 % of an extract with a T of 14·1) liberated much more enzyme than 10 % of the stable alcoholic extract of pig, and the glycerin extract of kept pig, though these extracts had tryptic values of respectively 19·0 and 28·4.

It may be asked how the enzyme liberating action of extracts can be connected with their stability as regards sodium carbonate. On a physical hypothesis of the action of enzymes an explanation is much easier than on a chemical. Thus if we suppose that an enzyme acts upon a zymogen by transmitting to it certain physical vibrations which it itself possesses, and if we imagine that the more pronounced these vibrations, the more sensitive is the enzyme to the action of sodium carbonate, then it would naturally follow that an extract containing a certain amount of highly unstable enzyme would have a greater enzyme liberating capacity than an extract containing an equal quantity (as far as its proteolytic action is concerned) of more stable enzyme. The enzyme liberating capacity would also, of course, depend on the actual number of enzyme molecules present, and so it is no more than one would expect for an unstable extract of pig's pancreas, with a T of 141, to have a greater liberating capacity than an even more unstable extract of sheep's pancreas, with a T of only 60.

To return to the consideration of the above table, the numbers in the extreme right-hand column indicate the (corrected) rennetic values induced in the glycerin extract after $1\frac{1}{4}$ hours at 38° (instead of an hour). If one glances down this column it will be seen that the values

follow one another in order of magnitude with only one or two minor exceptions. The column of R values of the added extract is exceedingly irregular, however, and shows but slight correspondence to the induced values column. The most glaring exceptions are noticed in the case of the glycerin extracts of kept sheep and pig's pancreas, for these extracts had rennetic values of respectively 378 and 310, but had not much more liberating power than other extracts with an R of less than 100. On the other hand, glycerin extract of woman's pancreas, with an R of only 100, had a greater liberating power than the alcoholic extract of pig's pancreas, with an R twice as great. Now the column of induced T values was purposely arranged according to the magnitude of these values, and hence we are driven to the conclusion, already arrived at, that the rennet ferment is liberated from its zymogen by the same agent that liberates the tryptic ferment from *its* zymogen, and this we know to be the tryptic ferment itself. Thus as regards the specific instances mentioned, the glycerin extracts of kept pancreas had only a moderate tryptic power, and the ferment they contained was comparatively stable, whereas the glycerin extract of woman's pancreas contained highly unstable trypsin. However, the glycerin extract of sheep's pancreas forms a slight exception, for though powerful as compared with the other extracts, it had not so proportionately great a capacity for liberating rennet ferment as the glycerin extracts of woman and pig's pancreas. Still, taking the observations as a whole, there can be no doubt that the liberating power of the trypsin molecules on the rennet zymogen depends both on their degree of instability, and on their absolute number, just as in the case of the tryptic zymogen.

Still other evidence in support of this relationship between the rennet zymogen and the tryptic enzyme is afforded by the results obtained with kept extracts. In those of the sheep's pancreas, we saw that the normal saline extract reached its maximum T after two days, but its maximum R only after eight days, whilst the glycerin extract reached its maximum T after 63 days, and its R only after 86 days. Again, in the case of the pig's pancreas extracts, the alcoholic extract reached its maximum T after nine days, and its R after 71 days; the normal saline extract its maximum T after five days, and its R after 16 days; the glycerin extract its T after 71 days, and its R after 105 days. There is an obvious delay on the part of the rennet enzyme therefore, and this we must suppose to be due to its liberation being dependent on the previous liberation of the tryptic enzyme.

Other observations on the liberating effects of active extracts on the zymogen of inactive extracts were made in the case of ox pancreas extracts, but in their case only the rennetic values were determined. The addition of both 1 and 10 % of normal saline and of alcoholic extracts (which had rennetic values of respectively 198 and 180) was found to induce an R of over 200 in aqueous extract of ox pancreas, in 20 hours. This aqueous extract itself had an R of 24·3, and spontaneously increased this value to 36·0 in the 20 hours. Again, the addition of 10 % of the same normal saline and alcoholic extracts to a saturated saline extract of ox pancreas induced rennetic values of respectively 176 and 136 in 20 hours. This saturated salt extract itself had an R of 18·2, and spontaneously increased this value to 23·0 in the 20 hours.

*The effect of keeping the gland tissue before extraction.*

It was shown by Heidenhain in his original memoir that if pancreatic gland tissue were exposed to the air some hours before extraction, it invariably contained free enzyme, whilst tissue extracted immediately after death contained little or none. Subsequent observers have confirmed this result, but have extended our knowledge very little further than the point at which Heidenhain left it.

In the case of almost every gland of which I have made extracts I have kept a portion of the minced tissue for 20 or more hours exposed to the air in a moist chamber before adding the extracting liquid. All such extracts, with perhaps one exception, showed a greater or less amount of ferment activity from the first. As regards the rennet ferment, it was found that a saturated salt extract of pig's pancreas, kept 19 hours before adding the extracting liquid, had an R of 10·0 after seven days' extraction. After the same period the saturated salt extract of fresh pancreas had an R of only ·9. Four days later, however, this latter extract had attained to three or four times the rennetic value of the former extract, and it finally after 130 days reached a maximum of 320, or more than treble the maximum value of the extract of kept gland. The curve of this extract of kept gland is given in Fig. 2. In the case of the dog's pancreas, normal saline extracts were made of portions of the gland substance after it had been kept respectively 28 and 70 hours. The extract of 28 hours' kept gland, when examined the next day, had an R of only 1·7, or less than the saline extract of fresh gland substance, which had an R of 3·2. Of course in this case the extraction has been going on for two days, instead of one, but still

the enzyme must have been developing more rapidly than in the kept gland. Ten days later, the extract of kept gland had attained an R of 4·50, or a higher value than any of the other extracts of this pancreas ever reached, so in this case the exposure to the air had a permanently favourable influence on the liberation of the enzyme. However, as can be seen in Fig. 4, the extract of gland kept 70 hours before extraction did not reach so high a value as any of the other extracts, so in this case, as in that of the pig, exposure of the gland destroyed some of the ferment. It should be borne in mind that these observations on dog's pancreas were made in October, whilst those on pig's pancreas, and the ones to be immediately described on ox pancreas, were made in August, when 20 hours' exposure would probably be equivalent to 40 or more in the colder month. The ox gland substance was exposed for 60 hours, and the normal saline extract of it, when examined two days later, had an R of 12·0. The similar extract of fresh gland substance had an R of 1·2, so the exposure had a distinct liberating effect on the zymogen. It had also a distinct destructive effect, for, as can be seen in Fig. 1, the maximum value attained by the extract of kept gland was only little more than a third of that of the similar extract of fresh gland.

In the case of the human pancreases examined, the condition of affairs was somewhat different, in that the glands remained for some hours untouched in the body. The first pancreas was obtained from a man, aged 21, who died four days after an accident. It was therefore nearly normal. The extracts were made seven hours after the gland had been removed from the body, and this was not done till 29 hours after death. A portion of the minced gland was exposed to the air for 13 hours before extraction, so this extract was one of gland substance kept 49 hours after death. The results obtained with these extracts are given in the accompanying figure. Here one can see that all the extracts except the glycerin ones, had reached their maximum rennetic values when they were first examined, viz., after two days' extraction. From this time onwards these values rapidly dwindled away, the aqueous extract deteriorating most rapidly, as usual. These results might of themselves be taken to indicate that the treatment this gland had undergone before extraction was sufficient to liberate all the enzyme it contained. The observations on the glycerin extracts, however, show that but little of the conversion of zymogen into enzyme can have occurred until after the extracting liquids had been added. Thus the concentrated glycerin extract had no rennetic power after six days' extraction, and only a very slight one

after 13 days. Now it was found by Kühne[1] that trypsin is insoluble in concentrated glycerin, so it was possible that in the present

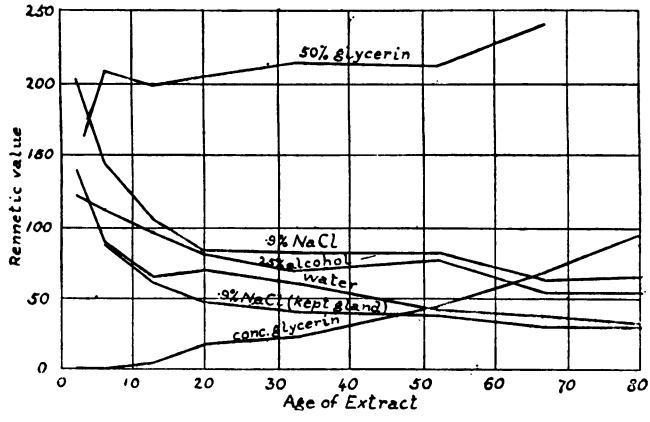


Fig. 8.

case the ferment actually existed in the gland tissue, but could not be extracted by the glycerin until this had, in virtue of its hygroscopic powers, attracted some moisture from the air, and so become slightly more diluted. As the filtering of glycerin extracts was always a slow process, and as any excess of filtrate not needed for experiment was returned to its stock bottle, there was doubtless a very slight dilution of the glycerin in this way. However, the glycerin extract undoubtedly contained a considerable quantity of zymogen, for after two days' extraction a portion of it, together with its gland substance, was diluted with an equal volume of water. Two hours after the dilution some of the extract was filtered off, and was now found to have an R of 22·6 (this value being multiplied by 2 to render it comparable to the other extracts). A few days later, some of this filtered extract, which had been kept separate, was found to have attained an R of 52, and this increase can only have been due to the conversion of zymogen.

The other human pancreas examined was that of a woman, aged 39, who had died of diabetes. The pancreas, obtained eight hours after death, was very tough and stringy. After 16 hours' extraction, the normal saline extract was found to have an R of 21·9, the alcoholic extract one of 3·5, and the 75 % glycerin extract one of 3·1. It is

[1] *Verhandl. d. naturh. med. Ver. zu Heidelberg*, Vol. I. p. 196. 1876.

probable, therefore, that the gland contained appreciable quantities of free ferment before the extracting liquids were added. The normal saline extract attained its maximum value of 61·0 after 12 days, and then gradually deteriorated; the alcoholic extract its maximum of 57·8, also after 12 days; and the glycerin extract its maximum of 100 after nine months.

As regards the tryptic ferment, the values given in the table on p. 278 show that the 75 % glycerin extract of sheep's pancreas (kept 63 hours before extraction) had attained a tryptic value of 9·0, after one day's extraction. As the maximum value this extract subsequently attained was only 18·2, the exposure of the gland substance must have set free a fair amount of ferment, though of course it is impossible to say how much was liberated before the addition of the extracting liquid, and how much after. It also destroyed a large amount, for the glycerin extract of fresh gland substance attained just four times as great a tryptic value. On the other hand, the rennetic value of this extract of kept gland, after five days' extraction (or eight days after removal of the glands from the body), had reached the high figure of 367, an amount little inferior to the maximum of 420 it subsequently attained. As in the case of the dog's pancreas, exposure of the gland substance to air before extraction yielded an extract with a higher rennetic value than was attained by any of the other extracts, the maximum for the alcoholic extract being 399, and for the glycerin, 346. This fact, taken in conjunction with the destruction suffered by the tryptic ferment, caused the ratio of R to T to average, over the whole period of observations, the extremely high figure of 33·0. These observations were started at the beginning of April, when the temperature was fairly low, but after its 63 hours' exposure the gland substance, as testified by its smell, had undergone a considerable amount of decomposition.

The pig's pancreas, on which tryptic estimations were made, was kept 25 hours before extraction with 75 % glycerin. A day later the extract had attained a T of 6·1, and an R of 109, but subsequent to this the ferments only slowly increased in activity. As can be seen in the table given on p. 280, the tryptic value did not reach its maximum of 35·0 for 135 days, and the rennetic, its maximum of 359 for 105 days. The maximum T attained by the glycerin extract of fresh gland was 208, and the maximum R, 512, hence the exposure of the gland might be taken to have destroyed about a third of the rennetic ferment, and about five-sixths of the tryptic. However, the observations on the

destruction rate of this extract by sodium carbonate show that such a deduction is not admissible. For it was found that even after 59 days' extraction, the extract kept for an hour at 38° with $Na_2CO_3$ increased in tryptic power by 37·6 %, so it must have contained considerable quantities of unconverted zymogen. Probably it contained a certain amount of zymogen even after 135 days' extraction, for the destruction rate was then only about 10 %, or considerably less than one would expect from the destruction rates exhibited by the alcoholic and saline extracts. The presence of unconverted zymogen was probably responsible for the very variable tryptic values exhibited by this extract of kept gland substance. Thus this value fell from 26·4 after 71 days' extraction to 16·5 after 105 days, and then rose again to 35·0 after 135 days. Presumably some very slight differences in the conditions of filtration of the extract, or of the subsequent digestion, brought about the conversion of variable quantities of zymogen into enzyme, and so gave variable results. The glycerin extract of kept sheep's pancreas gave equally variable values, the T falling from 18·2 after 86 days' extraction to 8·57 after 136 days, and then rising again to 13·5 after 149 days. However, the destruction rate of this latter extract kept fairly constant, and as it was also fairly high, there could not have been very much unconverted zymogen present. Why any zymogen at all should remain unconverted, as was the case not only in these glycerin extracts of kept gland substance, but also in the saline extracts of sheep and pig's pancreas, and alcoholic extract of pig's, seems very curious. Possibly the change of zymogen into enzyme is to a very slight degree a reversible one.

A few observations were made on other pigs' pancreas, the gland substance being extracted with 75 % glycerin after 20 hours' exposure to air. The values obtained are given in the table on p. 283. Here we see that after one day's extraction, the extract had a T of only 3·0, and an R of only 37·8. These values gradually increased, but even after 41 days' extraction had attained only about treble these amounts. The extract doubtless contained large quantities of unconverted zymogen, for after 21 days' extraction, the T of the extract increased by 51·6 % after keeping with $Na_2CO_3$ at 38°, and after 41 days' extraction, it increased by 14·5 %.

The evidence as to the effects of keeping the gland substance before extraction is thus somewhat variable and conflicting. Probably as a rule, if not invariably, the exposure to the air brings about the liberation of only a small quantity of enzyme, and the enzyme so liberated induces

further conversion of the zymogen into enzyme after the addition of the extracting liquid. Doubtless the liberated enzyme could act to some extent on the zymogen with which it was in contact before the addition of the liquid, and so set free more and more ferment, but I think it is very probable that most of the ferment so set free is quickly destroyed. Thus we saw that, with two exceptions, the extracts of kept gland substance were much feebler in rennetic power than the corresponding extracts of fresh gland substance, and very much feebler in tryptic power.

*The influence of acids on enzyme formation.*

It was pointed out by Heidenhain that the tryptic enzyme could be set free by the addition of weak acids to the gland tissue. The method suggested by him (*loc. cit.* p. 586) was to keep the minced gland for 10 minutes with an equal weight of 1 % acetic acid, and then add 10 volumes of glycerin to the mixture. Though I tried this method twice, both times with pig's pancreas, I met with no success. On one occasion the extract had no tryptic or rennetic power whatever, even after 21 days' extraction, and on the other occasion it had a rennetic value of 8·4 after one day's extraction, but showed no subsequent increase of ferment power. What may be true for the pancreas of the dog, which Heidenhain employed, may not hold, therefore, for the pancreas of other animals. Other observations, to be described later, lead me to think that the concentrated glycerin may of itself be sufficient to check the development of ferment activity.

Another method of acidification proved more successful. In this the gland tissue (pig's pancreas) was kept for 20 hours with an equal volume of ·2 % acetic acid, and was then mixed with three volumes of glycerin. A 20 % extract in 75 % glycerin (+ ·04 % acetic acid) was thus obtained. This yielded the following values:

| | 2 days | 4 days | 12 days | 21 days | 41 days |
|---|---|---|---|---|---|
| T | 4·4 | 4·6 | 8·8 | 13·2 | 18·0 |
| R | 44 | 67 | 80 | 83 | 120 |
| R÷T | 10·0 | 14·6 | 9·1 | 6·3 | 6·6 |
| % dest. | | *+62·0* | | *40·3* | *27·5* |

Here we see that after one day's extraction (or two days after the gland was removed from the body), the extract had a T of 4·4, and an R of 44. After 41 days' extraction these values had increased to respectively 18·0 and 120, but even then they were not half as great as the values obtained with the alcoholic extract of the same gland substance (cf. table on p. 283).

The addition of small quantities of acetic acid to diluted glycerin extract was found to hasten enzyme formation, provided that some of the gland substance were also present. The accompanying figures show that the addition of ·25 % acetic acid caused an appreciable increase in the rate of trypsin formation in four days, and a much more marked increase in nine days. The addition of ·05 % acetic acid seemed to

| | 2 days | 4 days | 9 days | 12 days |
|---|---|---|---|---|
| Concentrated glycerin ext. pig + gland subs. + 1 vol. water | — | 1·1 | 1·5 | 9·5 |
| ,, ,, ,, + ·05 % acetic acid ... | ·6 | ·9 | 5·7 | 33·3 |
| ,, ,, ,, + ·25 % ,, ... | ·9 | 1·6 | 5·9 | 25·1 |

produce no effect at first, but after 12 days it was apparently more successful than the stronger acid.

Probably the acid acts only indirectly by liberating something from the gland tissue which is able to excite enzyme formation, for if acid be added to the filtered extracts, it never, in my experience, hastens the conversion of zymogen into enzyme. For instance, a 75 % glycerin extract of pig's pancreas, diluted with twice its volume of water, was found to have developed a T of ·6 when kept at 38° for an hour. When ·01 % of HCl was added, it developed a T of only ·4, and when ·04 % HCl, it developed no tryptic power at all. On keeping some of this same diluted extract at 38° for an hour with 1 % of active glycerin extract of pig's pancreas (which had a T of 141), and various percentages of acid, the following values were obtained:

| | 1 hr. at 38° |
|---|---|
| 75 % glycerin ext. + 2 vols. water + 1 % active extract | T = 13·6 |
| ,, ,, ,, ,, ,, + ·001 % HCl ... | 13·7 |
| ,, ,, ,, ,, ,, + ·003 ,, ... | 12·8 |
| ,, ,, ,, ,, ,, + ·01 ,, ... | 12·3 |
| ,, ,, ,, ,, ,, + ·04 ,, ... | 3·3 |

That is to say, the addition of ·001 to ·01 % HCl had little or no effect, whilst the addition of ·04 % lowered the tryptic value induced to a quarter of its normal amount. Another series of observations made with another 75 % glycerin extract of pig's pancreas, but with the addition of 5 % active extract, gave the following results:

| | 1 hr. at 38° | 3 hrs. at 38° |
|---|---|---|
| 75 % glycerin ext. + 2 vols. water + 5 % active extract | 23·4 | 31·9 |
| ,, ,, ,, ,, ,, + ·01 % hydrochloric acid | 16·3 | 25·3 |
| ,, ,, ,, ,, ,, + ·05 acetic acid | 17·0 | 21·1 |
| ,, ,, ,, ,, ,, + ·2 ,, ,, | 12·5 | 17·8 |
| ,, ,, ,, ,, ,, + ·05 sodium carbonate | 24·3 | 29·2 |
| ,, ,, ,, ,, ,, + ·2 ,, ,, | 14·9 | 17·4 |

Here we see that ·01% HCl proved rather more harmful than before, it reducing the enzyme formation during the first hour by about a third. ·05% acetic acid had about the same effect, whilst ·2% acetic acid reduced the enzyme formation by nearly a half. The two experiments made with sodium carbonate show that ·05% of the alkali had no effect at all, whilst ·2% had about as much harmful effect as ·2% acetic acid. The values obtained after keeping the extracts for three hours at 38° are in fair agreement with those obtained after one hour, but as they seem to show a (proportionately) somewhat greater depreciation, one may conclude that the acids and alkali acted more by destroying the enzyme after it was formed, than by merely retarding its formation.

More elaborate series of observations were made on the influence of acids on the rennet zymogen, but, as already mentioned, this influence was always a retarding one. The accompanying table shows the results obtained by adding various percentages of hydrochloric, acetic and lactic acids to filtered normal saline extract of ox pancreas. The dilution attendant on the addition of acid was allowed for in calculating the rennetic value. It has been shown in a former paper that slight variations in the acidity are of considerable influence on the metacasein reaction, so in each determination the amount of sodium carbonate necessary for exact neutralisation of the extract to be added was run into the diluted milk. When the rennetic value was 200 or so, this was not necessary, the amount of extract used being so very small. For the sake of comparison, there are given in the first line of the table the rennetic values of some of the extract which was kept with its gland substance, and filtered off fresh as required; and in the second line, the values of some of the filtered extract to which no acid had been added.

| Percentage of acid | 2 days | 3 days | 5 days | 7 days | 10 days | 15 days | 23 days | 34 days | 70 days |
|---|---|---|---|---|---|---|---|---|---|
| Extract (fresh filtered) | 6·0 | 7·8 | — | — | *203* | 194 | 203 | 207 | 84·0 |
| ,, (kept filtrate) | 6·0 | — | — | — | 15·9 | — | *222* | — | 129 |
| ,, + ·035 HCl | — | 22·6 | 19·0 | 11·7 | 14·9 | 19·7 | 23·0 | 23·3 | *298* |
| ,, ·0247 ,, | — | 17·0 | 13·8 | 17·0 | 17·4 | 33·9 | 36·1 | *202* | 271 |
| ,, ·014 ,, | — | 13·0 | 10·8 | 11·1 | 14·3 | *264* | 275 | 210 | 182 |
| ,, ·0065 ,, | — | 13·4 | 7·4 | 17·3 | 12·4 | 30·1 | 30·8 | *231* | 141 |
| ,, ·40 acetic acid | 12·5 | — | 8·5 | 7·9 | 10·8 | 11·1 | 10·8 | 13·4 | 12·9 |
| ,, ·193 ,, | 11·9 | — | 9·1 | 12·4 | 15·8 | *297* | 283 | — | — |
| ,, ·083 ,, | 11·6 | — | 10·9 | 15·0 | 18·5 | 22·5 | *285* | 251 | — |
| ,, ·412 lactic acid | — | 15·2 | 7·4 | — | 5·0 | 7·5 | 8·1 | 14·2 | 12·9 |
| ,, ·215 ,, | 17·7 | 9·9 | 7·6 | — | 11·9 | 13·0 | 10·7 | *283* | 211 |
| ,, ·082 ,, | 15·8 | 12·5 | 14·8 | — | 15·4 | 16·0 | 17·1 | 23·7 | *195* |

From this table we see that the filtered extract containing no acid developed its rennetic power some time between the 10th and 23rd days of experiment, whilst the extract to which ·014% HCl had been added developed it some time between the 10th and 15th days. Probably in this case, therefore, the time of rapid enzyme formation was about the same, though from lack of sufficient observations one cannot speak for certain. However, the extract kept with its gland substance had developed its full activity by the 10th day, so there was an obvious retardation in the acidified extract as compared with this one. The other percentages of hydrochloric acid experimented with were less favourable, the extracts containing ·0247 and ·0065% developing their activity between the 23rd and 34th days, and that containing ·035%, not until after the 34th day. In the case of the acetic acid observations, we see that ·193% of acid acted as quickly as the most favourable concentration of hydrochloric acid, whilst ·083% of acid did not permit the enzyme formation to occur till a few days later. In the presence of ·40% acetic acid, and also ·412% lactic acid, there was no conversion of the zymogen even after 70 days. The other two concentrations of lactic acid tried were also distinctly unfavourable, one of them not permitting of enzyme formation till between the 23rd and 34th days, and the other, only between the 34th and 70th days.

Taking the results as a whole, therefore, we see that in the presence of various percentages of acid, the extract retained a nearly constant rennetic value for 10, 15, 23 or 34 days, and then suddenly developed its full enzyme power. It seems as if the addition of the acid always, or nearly always, checked the tendency to the conversion of the zymogen into enzyme, but that this check could succeed only for a time. Ultimately the zymogen accommodated itself to its abnormal conditions, and proceeded to undergo its conversion.

A somewhat unexpected feature of these observations is the stability of both zymogen and enzyme in the presence of acid. Even ·035% HCl, acting for more than 34 days, had absolutely no destructive effect upon the zymogen, as the enzymic power developed after that date was greater than was observed in any other case. Also the enzyme, once liberated, appeared to be more stable in the presence of small quantities of acid than if no acid had been added at all. It should be mentioned, however, that the extracts are not strictly comparable, for those containing acids were all diluted more or less by the addition of various amounts of standard (dilute) acid, whilst the unacidified extract was not diluted at all.

Arguing from the observation of Langley and Edkins, that ·1% HCl would effect an almost complete conversion of pepsinogen into pepsin in a minute, a further series of observations was made on more powerfully acidified extract. The extract used was an aqueous one of ox pancreas. The results obtained are given in the accompanying table. The values given in the first line indicate the development of rennetic power in the extract left standing with its gland substance, no observations being made on the filtered (unacidified) extract.

| Percentage of acid | Immediately | after 6 hrs | 1 day | 3 days | 8 days | 16 days | 70 days |
|---|---|---|---|---|---|---|---|
| Aqueous extract ... | 21·4 | — | 24·3 | 57·0 | *216* | 200 | 35·4 |
| + ·10 % HCl ... | 21·9 | 25·8 | 25·4 | 24·2 | 20·3 | 23·4 | 31·2 |
| ·0577 ,, ... | — | 25·8 | 20·0 | 22·7 | 26·0 | 28·7 | *254* |
| ·0345 ,, ... | — | — | 19·8 | 25·7 | 39·6 | *106* | 197 |
| ·0345 ,, (at 38°) | — | 26·4 | 33·7 | 8·9 | 5·1 | 5·0 | — |

These figures show that the addition of ·1% HCl had no effect either immediately (*i.e.*, about a minute after the addition of the acid), or after 6 hours, or even after 70 days. However, ·0577% HCl permitted the enzyme formation to take place some time between the 16th and 70th days, whilst in the case of ·0345% HCl, the zymogen was in the middle of its conversion after 16 days' acidification. We see that the extract kept with its gland substance had undergone a certain amount of conversion after 3 days, and had completed it after 8, so here again, as in the former series of observations, the acid had only a retarding influence.

It will be seen that ·1% HCl, though it did not allow of enzyme formation, yet had no destructive influence on the small quantity of enzyme already present, even after 70 days. This and the other similar but less striking results contained in the former table seem at first sight highly contradictory to the tryptic determinations, for in these we saw that ·04% HCl, and ·2% acetic acid, had a markedly destructive action on the ferment. Also Kühne[1] found that hydrochloric acid above ·05% in strength was injurious to trypsin, and Langley[2] found that a glycerin extract of pig's pancreas lost a considerable amount of its proteolytic power, if kept at 38° for 2½ hours with ·05% HCl. On the other hand Engesser[3] found that the proteolytic power of a dried pancreas preparation was undiminished after two hours' digestion at

[1] *Verh. Naturhist.-med. Vereins zu Heidelberg*, I. p. 193. 1877.
[2] This *Journal*, III. p. 262. 1881.
[3] Maly's *Jahresb.* 1880, p. 297.

40°—50° with gastric juice containing ·5% HCl. He attributed this remarkable result to the preparation containing only zymogen, and no enzyme, but this explanation is not accepted by Ewald[1], or by Langley (*l. c.*).

It should be borne in mind, however, that what holds for room temperature does not necessarily hold for a temperature of 38°. In the last line of the above table are given the rennetic values of some of the extract kept at 38°, instead of room temperature, with ·0345% HCl. In this case there was a slight amount of enzyme formation up to the end of the 1st day, but after this the ferment power rapidly deteriorated. Unfortunately no observations were made upon the unacidified extract kept at 38°, so one cannot say for certain whether the presence of the acid increased the rate of destruction of the ferment. Again, one must remember that the rennet ferment is a distinctly more stable body than the tryptic, so one cannot argue very closely from the results obtained with one ferment to those obtained with the other.

Another argument in favour of the comparative stability of trypsin in the presence of acid at room temperature, is afforded by the case of the active pancreatic extracts themselves. In these extracts the natural acidity is quite considerable, and yet we have seen that as a rule they deteriorate only slowly in enzymic power. To estimate this acidity, series of titrations were made with ·2 % $Na_2CO_3$, using phenol phthalein as an indicator. The figures given in the accompanying table indicate the number of c.c. of 1% $Na_2CO_3$ necessary to neutralise 100 c.c. of the various extracts of ox pancreas; but for economy's sake, and as great accuracy was not essential, the titrations were generally made with 1 c.c. of extract.

| | 1 day | 5 dys | 9 dys | 13 dys | 19 dys | 27 dys | 36 dys | 45 dys | 62 dys | 84 dys |
|---|---|---|---|---|---|---|---|---|---|---|
| Aqueous extract ... ... | 9 | 26 | 39 | 39 | 41 | 39 | 46 | 47 | 51 | 63 |
| ·9% NaCl ,, ... ... | 9 | 20 | 22 | 24 | 34 | 40 | 51 | 51 | 59 | 64 |
| ,, ,, (kept gland) | — | 32 | 31 | 32 | 33 | 31 | 36 | 38 | 41 | 40 |
| Sat. NaCl ,, ... ... | 16 | 21 | 29 | 30 | 31 | 46 | 65 | 63 | 60 | 71 |
| Alcoholic ,, ... ... | 11 | 50 | 60 | 60 | 60 | 55 | 55 | 61 | 56 | 53 |

The continuous lines drawn between certain of the values indicate when the most rapid formation of rennetic ferment was occurring (*vide* the curves in Fig. 1), whilst the dotted lines indicate when the ferment was undergoing its most rapid destruction. As far as one can see, the

[1] Maly's *Jahresb.* 1880, p. 297.

changes in acidity have little to do either with the rise or the fall of enzymic power. In the case of the aqueous extract, the acidity remained practically constant from the 9th day until the 27th, and it was only after the period of most rapid ferment liberation that the acidity began to rise again. In the normal saline extract the period of most rapid ferment formation was accompanied by a moderate increase of acidity, but there was a still greater increase after the ferment formation had ceased. In fact, it was found that in almost all of the various extracts of the various pancreases examined, the most considerable rise of acidity followed, or accompanied, the period of most rapid enzyme formation, and did not precede it. This was almost certainly owing to the auto-digestion of the gland substance which ensued after the ferment was liberated. Thus filtered extracts showed no increase of acidity either before, during, or after the time at which their zymogen was converted into enzyme. For instance, some of the normal saline extract of ox pancreas was filtered off after seven days' extraction, it then having an acidity of 20, and a rennetic value of 4·0. Twenty-nine days later, its rennetic value was 222, but its acidity was unaltered.

The normal saline extract of kept pancreas is of some interest, for we see that though at the beginning it had a considerably greater acidity than the similar extract of fresh gland, yet it did not ultimately attain to nearly so high a value, presumably because its low ferment activity did not effect so much auto-digestion. The saturated salt extract showed a considerable increase of acidity just at the time of enzyme formation, but the alcoholic extract developed a large acidity during the first few days of extraction, or before the period of rapid enzyme formation. A somewhat similar rise of acidity before enzyme formation was observed in two other alcoholic extracts, so this is evidently in some way dependent on the nature of the extracting liquid.

It seems unnecessary to quote the acidity values obtained with the extracts of pig, sheep, dog, and human pancreases, as they teach one nothing more than can be learnt from these ox pancreas values. The final acidity values of the extracts, the last time they were tested, are given in the accompanying table. The rennetic values of the extracts, at or about these times, can be determined by reference to Figs. 2, 3, 4, and 8.

| | Pig | Sheep | Dog | Man |
|---|---|---|---|---|
| Age of extracts in days | 90 | 115 | 50 | 67 |
| Aqueous extract | 45 | 55 | 61 | 47 |
| NaCl ,, | 76 | 61 | 53 | 40 |
| Alcohol ,, | 43 | 64 | 76 | 44 |
| Glycerin ,, | 46 | 45 | 52 | 76 |

These values are obviously variable, and do not seem to bear any near relationship either to the nature of the gland substance, or to the nature of the extracting liquid. The NaCl values of the pig and sheep's glands are for saturated salt extracts, and of the other two glands, for normal saline extracts. The average acidity of all the extracts is 55, and supposing it were entirely due to lactic acid, it would follow that the extracts contained on an average ·47 % of this acid. Thus with phenol phthalein as indicator, in cold solution, two equivalents of $Na_2CO_3$, or 106 parts by weight, would be neutralised by one equivalent of a weak organic acid (*e.g.* 90 parts of lactic acid). With acids of greater molecular weight than lactic acid, the percentage present would of course be correspondingly larger. Now we have seen that saturated salt solution preserved the rennetic ferment better than almost any of the other media employed, and the average acidity of these extracts was no less than 69 (= ·59 % of lactic acid). If this very large amount of organic acid is practically without influence, therefore, it is not surprising that ·1 % of hydrochloric acid should also be found innocuous.

As regards the tryptic ferment, we saw that all the extracts retained considerable activity for days or even months, and as the acidities of these extracts must have been very similar to those of the ox pancreas extracts above recorded, it follows that the tryptic ferment is likewise fairly stable in the presence of considerable amounts of organic acid.

*The influence of products of digestion on enzyme formation.*

We saw that the addition of 1 % of active glycerin extract of pig's pancreas to 1 c.c. of inactive extract mixed with fibrin and ·4 % $Na_2CO_3$ was able, during the course of digestion (*i.e.* during 20 to 30 minutes), to liberate enzyme giving a T of 8·4, whilst if the active and inactive extracts were previously kept together for an hour at 38°, the T induced was only 4·4 in excess of this value. Apparently, therefore, products of digestion may markedly stimulate the conversion of zymogen into enzyme. In order to test the matter further, two digestion liquids were prepared by keeping equal volumes of fibrin with ·05 % $Na_2CO_3$ and respectively 1 and 10 parts of pancreatic extract for two days at 38°. Both liquids were slightly acid at the end of this time, and in the one containing the smaller amount of extract, only about half of the fibrin had dissolved. Various volumes of these quick and slow digest liquids were added to diluted glycerin extract of pig's

pancreas, and kept for an hour at 38°. The tryptic values so induced were as follow:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 c.c. glycerin ext. pig | + 2 | c.c. water | 1 hr. at 38° | ... | | | T = | ·64 |
| ,, ,, | 1·7 | ,, | + ·3 | c.c. | slow | digest | | ·66 |
| ,, ,, | 1·0 | ,, | 1·0 | ,, | ,, | | | ·85 |
| ,, ,, | 1·9 | ,, | ·1 | ,, | quick | digest | | 1·5 |
| ,, ,, | 1·7 | ,, | ·3 | ,, | ,, | | | 3·1 |
| ,, ,, | 1·0 | ,, | 1·0 | ,, | ,, | | | 7·0 |

The slow digest liquid had thus a very slight liberating action on the zymogen, whilst the quick digest liquid had a fairly considerable one, especially when 1 c.c. of it was added to 1 c.c. of inactive extract.

Another series of observations was made in which 1 % of active glycerin extract of pig's pancreas was added to the inactive extract, in addition to the digest liquids. The following results were obtained:

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 c.c. glycerin ext. pig | + 1 % active ext. | + 2 | c.c. $H_2O$ | 1 hr. at 38° ... | ... | | | | T = | 13·6 |
| ,, ,, | ,, ,, | + 1·9 | ,, | + ·1 | c.c. | slow | digest | | | 12·2 |
| ,, ,, | ,, ,, | 1·0 | ,, | 1·0 | ,, | ,, | | | | 10·1 |
| ,, ,, | ,, ,, | 1·9 | ,, | ·1 | ,, | quick | digest | | | 13·2 |
| ,, ,, | ,, ,, | 1·0 | ,, | 1·0 | ,, | ,, | | | | 10·4 |

We see that in every case the tryptic value induced was less than when no digest liquid was added at all. This must have been due to the retarding action of the digest liquids on the digestive action of the liberated enzyme, for the addition of 1 c.c. of the quick digest liquid, which we saw to be much more efficacious in liberating enzyme than the addition of ·1 c.c., produced considerably more retardation. The tryptic ferment thus complies with the general rule as to the gradual inhibition of ferment activity by the collection of products of action.

Other observations were made with digestive liquids which had been kept for months, and also with putrefaction products, but in no case was any liberation of enzyme noticed. Probably, therefore, the liberating action depends on a few definite digestive products, and not on all of them. The subject was not pursued in any detail, but a few observations were made upon the influence of tyrosin, or what was regarded as a typical digestion product, and on creatin, a typical meat extractive.

On keeping 1 c.c. of glycerin extract of pig's pancreas with 2 c.c. of water and respectively ·1 and ·02 % of tyrosin, and ·1 and ·02 % of creatin, for an hour at 38°, tryptic values of respectively ·75, ·67, ·66 and ·63 were found to be induced. When only water was added, the T induced was ·64, so possibly the tyrosin may have had a slight enzyme liberating action. In any case, the creatin had none.

On keeping 1 c.c. of the diluted inactive extract with 1 % of active extract and respectively ·18, ·10 and ·02 % of tyrosin, tryptic values of respectively 14·6, 12·9 and 11·4 were induced; and when with ·18, ·10, ·10 and ·01 % of creatin, values of 12·3, 16·7, 14·9 and 12·2 were induced. When nothing was added, the tryptic value induced was 12·7, and hence it follows that in no case did the addition of tyrosin and creatin have much effect in either direction. As both of the observations with ·10 % creatin were found to give a slightly greater tryptic value than the normal, this result is probably a genuine one.

*The influence of dissolved gases on enzyme formation.*

It is stated by Podolinski[1] that if oxygen be bubbled through inactive glycerin extract of fresh gland containing 1·2 % $Na_2CO_3$ for ten minutes, this develops marked proteolytic power. Hydrogen, on the other hand, has no influence.

I endeavoured to repeat this observation by passing a current of oxygen (Brin's) through inactive glycerin extract of pig's pancreas diluted with twice its volume of 1 % $Na_2CO_3$. The extract was kept at 38°, and the gas was passed through for 15 minutes, but there was not the least increase of tryptic power. 1 % of active extract was then added to inactive extract diluted with two volumes of 1 % $Na_2CO_3$, and after 15 minutes of oxygen treatment at 38°, the tryptic value induced was found to be 12·4. Other extract in all respects similarly treated, except that no oxygen was bubbled through, gave a tryptic value of 9·8, hence the oxygen had a distinct enzyme liberating action.

In the other observations made, the glycerin extract of pig's pancreas was merely diluted with twice its volume of water, no sodium carbonate being added. The accompanying table shows the effects produced both by carbon dioxide and by oxygen, after bubbling these gases through the extract, kept at 38°, for respectively one and two hours. The carbon dioxide was passed into the extract from an iron bottle, a wash bottle of water being interposed.

| | 1 hour | 2 hours |
|---|---|---|
| Diluted extract (alone) ... ... ... ... | 1·1 | 1·4 |
| ,, ,, +oxygen ... ... ... | 1·2 | 1·5 |
| ,, ,, +$CO_2$ ... ... ... ... | 1·3 | 1·9 |
| ,, ,, +1 % active extract (alone) | 12·6 | 21·0 |
| ,, ,, ,, ,, ,, +oxygen | 18·0 | 21·7 |
| ,, ,, ,, ,, ,, +$CO_2$ | 11·8 | 20·3 |

[1] *Beiträge zur Kenntniss des pancreatischen Eiweissferments*, Breslau, 1876 (quoted by Heidenhain, *Hermann's Handbuch*, v. 1. p. 189).

We see that neither oxygen nor $CO_2$ had much influence on the inactive extract, the $CO_2$ apparently inducing a very small amount of enzyme formation. In the presence of 1 % of active extract, however, very different effects were produced. The oxygen now increased the tryptic value by 43 %, when bubbled through for an hour, whilst the $CO_2$ decreased it by 6·4 %. Probably, therefore, these gases act not so much by liberating or preventing the liberation of enzyme from zymogen, but rather by enabling the enzyme already present to act with increased or diminished efficiency. After two hours' treatment with the gases, the tryptic values do not show nearly so great differences. This is presumably because the maximum tryptic value which could be developed under such conditions (shown by other observations to be in this case about 30) was being approached.

A similar series of observations was made with another inactive 75 % glycerin extract of pig's pancreas, and with similar results. The values obtained are given in the accompanying table:

| | 1 hour | 2 hours |
|---|---|---|
| Diluted extract (alone) ... ... ... ... | ·61 | 1·3 |
| ,, ,, + oxygen ... ... ... | ·54 | 1·2 |
| ,, ,, + $CO_2$ ... ... ... ... | ·53 | ·9 |
| ,, ,, + 1 % active extract (alone) | 12·1 | 18·0 |
| ,, ,, ,, ,, ,, + oxygen | 15·5 | 16·9 |
| ,, ,, ,, ,, ,, + $CO_2$ | 10·6 | 16·5 |

Again, we see that with the inactive extract, neither oxygen nor $CO_2$ had much effect. The $CO_2$ seemed to slightly retard the enzyme formation, when acting for three hours, but in view of the previous result, in which apparently there was a slight acceleration, one must conclude that there is probably no influence either in one direction or the other.

The values obtained for inactive extract containing 1 % of active extract resemble those obtained before, as far as the 1 hour experiments are concerned. Thus the oxygen induced a tryptic value 28 % in excess of the normal, and the $CO_2$ one 12 % in defect. After passage of the gases for three hours, however, lower tryptic values were obtained than with the normal unaërated extract. Possibly this may have been due to the cumulative action of small quantities of impurities contained in the gases.

*Practical Conclusions.*

As active tryptic extracts are often required for laboratory purposes, it may not be out of place to indicate what appear to be the readiest methods of obtaining them. Heidenhain's method of treating the gland substance for a short time with acetic acid, and then adding concentrated glycerin, does not seem to be successful with the pancreas of the pig, whatever may be the case with that of the dog. Also we saw that exposure of the minced gland substance to the air some hours before extraction, though it yielded fairly active extracts, destroyed the larger part of the tryptic ferment present. Much more active extracts could be obtained with almost as great celerity by adding 1 part of methylated spirit and 3 parts of water to 1 part of minced gland. Several glands should be obtained and minced together, as if only a single gland be employed it may be several days before proteolytic activity begins to develop. Such alcoholic extracts attain their maximum activity in a week or a fortnight, and then gradually deteriorate. If permanently active extracts be desired, glycerin is the only extracting medium one can employ. If, however, concentrated glycerin be added to fresh gland substance, the zymogen may never undergo its conversion into enzyme. If glycerin containing 25 % of water be used instead, the formation of a very active extract is only a question of time, but in cold weather this time may extend to months. If glycerin containing 50 % of water be used, then the extract will probably become active in a few days or weeks. But another difficulty then presents itself. Once the activity has developed, auto-digestion takes place to such an extent that a clear extract can only be filtered off with extreme slowness. The greater the percentage of water added to the glycerin, the greater the degree of auto-digestion. In concentrated glycerin there is practically none, and filtration is easy, but as already stated, the use of concentrated glycerin may be absolutely fatal to success.

To obtain an exceedingly active glycerin extract of trypsin, the best plan would be to make an extract of pig's pancreas in 75 % of 50 % glycerin, and test its proteolytic power every few days. At first this would show scarcely any increase, and then more or less suddenly would begin to rise rapidly. After it had risen considerably, but before much auto-digestion had occurred, the glycerin should be filtered off from the gland substance, and kept separate. Its activity would probably increase to some extent after filtration, and then remain nearly constant for months and perhaps years. If the glycerin be filtered off from the gland substance before it has practically any

proteolytic power, then if a 75 % glycerin extract, it would probably develop its activity in course of time; if a 50 % glycerin extract, it would certainly do so before long. It would never acquire the activity of the extract filtered off from its gland substance after it had become active, however, for we have seen that much of the zymogen is insoluble, and is only rendered soluble and converted into its enzyme by the presence of active ferment.

To obtain an active glycerin extract in a few days, the following procedure might be adopted. Mix a small quantity of the minced gland substance with dilute alcohol, and the larger part with 50 % glycerin. When this alcoholic extract has been found to have become active, bring about the conversion of the zymogen of the glycerin extract into enzyme by the addition to it of a small quantity (1 to 10 %) of the active alcoholic extract. After a few days, when this addition of enzyme had induced the conversion of most of the zymogen, filter off the glycerin from its gland substance. Of course any active extract of any gland, whatever its origin, would do as well as the alcoholic extract of the same gland.

The pancreas of the pig should always be used in preference to that of the sheep, and that of the sheep in preference to that of the ox. Possibly dog's pancreas might yield rather more powerful extracts than pig's, but it cannot be obtained in such quantity. Also in diastatic activity it is considerably inferior to the pig's pancreas.

With a view to obtaining more definite information as to the rate of development of the tryptic enzyme in filtered glycerin extracts of various concentrations, a series of observations was made on the effects of adding respectively 1 and 10 % of active glycerin extract of pig's pancreas (which had a T of 140) to a concentrated glycerin extract of pig's pancreas, to a 75 % glycerin extract, and to 75 % glycerin extract diluted with ¼ and with ½ its volume of water. The values obtained are given in the accompanying table. These have been corrected for the T of the active extract added.

| | Tryptic value induced in inactive extract | | | | | | |
|---|---|---|---|---|---|---|---|
| | immediately | 1 day | 2 days | 4 days | 8 days | 16 days | 21 days |
| Concentrated glyc. ext. + 1 % active ext. | 5·7 | — | — | 8·5 | 9·9 | 9·5 | 12·1 |
| 75 % glyc. ext. + 1 % active extract | 7·4 | 11·5 | 11·8 | 14·6 | 15·5 | 16·8 | 21·3 |
| ,, + ¼ vol. water ,, | 7·4 | 16·4 | 22·6 | 33·5 | 47·7 | 51·5 | — |
| ,, + ½ vol. ,, ,, | 7·4 | 20·3 | 30·2 | 51·3 | — | 50·1 | — |
| Concentrated glyc. ext. + 10 % active ext. | 10·0 | — | 19·4 | 18·1 | 16·1 | 16·2 | 20·5 |
| 75 % glyc. ext. + 10 % active extract | 11·8 | 22·1 | 24·2 | 28·2 | 32·4 | 33·9 | 41·1 |
| ,, + ¼ vol. water ,, | 11·8 | 26·4 | 44·8 | 48·4 | 50·5 | 57·2 | — |
| ,, + ½ vol. ,, ,, | 11·8 | 38·8 | 52·1 | 64·5 | — | 58·7 | — |

Here we see that in the concentrated glycerin extract only a comparatively slight amount of zymogen had undergone its conversion even after 21 days. The figures in the column headed "immediately" indicate the (corrected) tryptic values obtained on adding the active extract to the inactive mixed with the fibrin and ·4 % $Na_2CO_3$. For some unknown reason this did not yield by any means so great a tryptic value as when the active and inactive extracts were mixed together before adding them to the fibrin and $Na_2CO_3$. Thus we see that in the case of this concentrated glycerin extract the immediate tryptic value with 10 % active extract was 10·0, whilst that obtained after the extracts had been kept in contact for two days was 19·4. This considerable rise could scarcely have been due to conversion of zymogen in the interval, for we see that there was no further rise of tryptic power even after an additional 14 days. Again, we see that in the 75 % glycerin extract to which 10 % active extract had been added, the tryptic value after 1 day had risen from 11·8 to 22·1, but in the next day rose only from 22·1 to 24·2, and in the next two to 28·2. The values obtained on addition of 1 % of active extract indicate the same thing, though not so distinctly. A possible explanation of this unexpected result lies in the extreme sensitiveness of the unstable trypsins in active extracts to $Na_2CO_3$. Some of the most unstable molecules, and therefore the most active as regards enzyme liberation, may perhaps be destroyed by the $Na_2CO_3$ before they have had time to come into contact with the zymogen molecules of the inactive extract.

To return to the discussion of the table, we see that in the 75 % glycerin extract the conversion of the zymogen was a great deal faster than in the concentrated glycerin, though with 1 % of active extract considerably less than half of the total amount present had undergone conversion in 21 days. With 10 % of active extract, however, about two-thirds had been converted in this period. The results obtained with the 75 % glycerin extract diluted with respectively $\frac{1}{4}$ and $\frac{1}{2}$ its volume of water show that the glycerin needs only a very small dilution in order to render the rate of conversion as rapid as one can desire, as far as regards the practical preparation of active extracts. Thus when either 1 or 10 % of active extract was added, the extracts so diluted reached what appeared to be their limiting values after respectively 16 and 4 days. The final values obtained on addition of 10 % of active extract are somewhat greater than those with 1 %, so presumably in this latter case small quantities of the zymogen remained permanently unconverted into enzyme.

When these observations were made, the average room temperature was about 14°. As we have already seen, the rate of conversion is very greatly increased with rise of temperature. If, therefore, it were desired to prepare active extracts as quickly as possible, they might be warmed to 20°—24°, but it would be very unsafe to keep them much above this temperature. Thus we have seen that at 38° the enzyme in diluted extract is destroyed as rapidly as it is formed, after the first three hours.

As regards the methods adopted for the estimation of the tryptic and rennetic ferments, that for the tryptic proved to be very reliable and convenient. It is obvious, however, that with very active and unstable extracts, as compared with stable, a large quantity of ferment would digest the fibrin relatively more quickly than a small quantity. Thus the shorter the digestion time, the less the amount of ferment destroyed before the digestion is completed. However, the average values given in the former paper for the determination of the T of an extract from any given digestion time were found to hold fairly well for all but the extremely active and sensitive glycerin extracts. For these extracts, another series of values was used, in which the increase of T for digestion times less than 30 minutes in duration, and the decrease for digestion times more than 30 minutes, was only about two-thirds of that given in the table. The differences in the results thereby produced were as a rule only very slight, for great care was taken to use such an amount of extract as would give a digestion time not far removed from the standard 30 minutes. Of course it was not always possible to anticipate even roughly the tryptic value of a given extract, so if the digestion time obtained departed much from the standard, another determination was made, using more, or less, extract, as the case might be.

The method adopted for estimating the rennet ferment unfortunately is not very reliable. The determinations would work out all right for days and even weeks, and then suddenly would all be 50% or so too high. Occasionally, in fact, the diluted milk would give a slight coagulum on boiling after keeping a short time at 38°, though no extract whatever were added. This was due, I believe, to the semi-sour milk over from the day before being mixed by the dairyman with the fresh morning's milk, for the morning's milk might be found useless for experiment, whilst the fresh afternoon's milk would give perfectly reliable results. Unless, therefore, one were to obtain one's milk from a single cow, in a state of absolute freshness and purity, the metacasein

reaction must always remain somewhat unreliable. It is easy to recognise when one's results are too high, for they are all of them increased by about the same relative amount. In such cases, therefore, they were all rejected, and were repeated the same afternoon, or the next day, with fresh milk.

## SUMMARY.

Extracts of a fresh pancreatic gland as a rule show no ferment activity for some days, and then more or less suddenly develop nearly their maximum power. For instance, the 25% alcoholic extract of an ox pancreas was found to have a rennetic value of 1·7 after 5 days' extraction, but one of 167 after 9 days; the normal saline extract of the same pancreas one of 7·8 after 13 days, and one of 200 after 19 days; the aqueous and saturated salt extracts, values of respectively 16·8 and 3·9 after 19 days, and of 200 and 295 after 33 days. After maintaining their maximum power for a few days or weeks, the extracts gradually deteriorate.

When the glands from several different animals are minced together and extracted, the ferment activity begins to develop at once. In the case of sheep's glands, the 25% alcoholic extract was found to attain its maximum tryptic (T) and rennetic (R) values in 12 days: in the normal saline extract its maximum T in 2 days, and its maximum R in 8 days: in the 75% glycerin extract its maximum T in 63 days, and its R in 86 days. In the case of pig's glands, the alcoholic extract reached its maximum T in 9 days, and its R in 59 days: the normal saline extract its T in 5 days, and its R in 16: the glycerin extract its T in 71 days, and its R in 105. The glycerin extracts attained more than twice the T of the alcoholic extracts, and 3 to 10 times that of the saline extracts, but the maximum rennetic values were comparatively little influenced by the nature of the extracting liquid. Still as a rule the tryptic and rennetic powers of extracts show a distinct resemblance, but this is only qualitative, not quantitative. The relation between the R and T values may vary considerably from day to day in the same extract, and still more so in different extracts. Also the extracts of pig's glands, as compared with those of sheep's, contain relatively much more tryptic ferment.

The destructibility of the trypsin, as tested by keeping the diluted extract for 1 hour at 38° with ·4% $Na_2CO_3$, and determining the percentage of ferment destroyed at the end of that time, varies con-

comitantly with the activity of the extract. Thus the normal saline extract of sheep, when at its maximum proteolytic power (T = 23·7), had 76·9% of its ferment so destroyed. When its T had sunk to 15·0, the destruction rate fell to 51·7%; when to 9·5, to 38·2%, and when to 5·7, to 19·6%. The other extracts of sheep and of pig's pancreas showed a similar increase of stability with decrease of tryptic power. The glycerin extract of sheep reached a (maximum) T of 72, and had no less than 80% of its ferment destroyed per hour. The glycerin extract of pig, with a T of 208, had only 69% destroyed, and the other extracts of pig were also slightly more stable than those of sheep, in spite of their having treble the tryptic power. Also the two glycerin extracts did not increase in stability even after their T had fallen off to respectively 47·9 and 139, hence probably about half of the trypsin they originally contained was of the same degree of instability.

If filtered glycerin extracts of zymogen be diluted with water, they spontaneously develop tryptic power at a rate increasing with the degree of dilution. If small quantities of an active extract be added in addition, the rate of conversion of zymogen into enzyme is enormously increased. For instance, the addition of 1% of active glycerin extract of pig's pancreas to inactive glycerin extract caused the T to increase from ·85 to 88·1 in 2 days; the addition of 3%, to 89·1 in 1 day; and of 10%, to 97·5 in 9 hours. When no active extract was added, the T increased only to 1·14 in 4 days. At 38°, instead of at room temperature, the rate of conversion is three or more times as rapid. On comparing extracts of pancreas of different animals, it was found that their enzyme liberating power depended on their tryptic activity, and on their degree of instability (as regards $Na_2CO_3$); but, proportionately to their tryptic activity, active extracts set free the enzyme from the zymogen much more rapidly than the comparatively inactive extracts.

The rennet ferment in inactive extracts is set free as well as tryptic, but it was found that the liberation of the ferment from its zymogen was effected, not by the rennet ferment, but by the tryptic. Thus if an extract containing a large amount of rennet ferment, and but little tryptic, were added to an inactive extract, there was but little liberation of either rennetic or tryptic ferment; but if one containing a small amount of rennet ferment and a large amount of tryptic were added, there was a considerable liberation of both ferments.

In support of Langley's view of the existence of zymogens of various degrees of elaboration, it was found that an inactive extract, on keeping, showed a considerably increased susceptibility to the enzyme liberating action of active extract. Also a considerable part of the zymogen in gland tissue only becomes soluble after the gland has been left in contact with active extract.

It was found that the amount of ferment liberated by exposure of the minced gland substance to the air for 20 or more hours was as a rule very small, most of the ferment being set free after the addition of the extracting liquid. As a rule, also, exposure to the air destroyed more than half of the rennetic ferment, and more than three-fourths of the tryptic ferment.

Heidenhain's method of keeping gland substance for 10 minutes with 1% acetic acid before mixing with glycerin did not prove successful in the case of pig's pancreas, but fairly active extracts were obtained by keeping the gland with ·2% acetic acid for 20 hours before adding the glycerin, and also by adding ·05—·25% acetic acid to diluted glycerin extract and gland substance. The addition of acids to filtered extracts never in any case hastened enzyme formation, but almost always considerably retarded it. Ultimately, however, the zymogen underwent conversion into enzyme, even in the presence of ·058% HCl, ·19% acetic acid, and ·21% lactic acid. The acid seemed to have no destructive effect on either enzyme or zymogen at room temperature, but this is not remarkable as the natural acidity of kept extracts, in which the ferments may remain undecomposed for months, may be equivalent to ·59% lactic acid.

The addition of small quantities of tryptic digestion products to inactive extracts was found to have a moderate enzyme liberating effect.

If oxygen or $CO_2$ were bubbled for 1 to 3 hours through diluted glycerin extract kept at 38°, the enzyme was not liberated any more rapidly or slowly than in unaërated extract. If, however, 1% of an active extract were added in addition, then in 1 hour the oxygen increased the enzyme formation by respectively 43 and 28% in two different observations, whilst the $CO_2$ decreased it by respectively 6·4 and 12%.

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