

remarkable structure only exists in the Acipenseroids; it is not found in *Polyodon*.

In the Selachians the "placoid" plates or spines are not brought under the influence of the chondrocranium, which has neither parosteal plates applied as splints to it, nor ectosteal plates grafted upon it.

In *Acipenser* there are both parostoses applied to the oral apparatus, and ectosteal centres in the post-mandibular arches; moreover, along the side of the skull, in old individuals, plates of bone appear as these splints or parostoses, that are manifestly the forerunners of the deeper plates that, in the higher Ganoids and the Teleostei, form the proper ectosteal bony centres of the more or less ossified cranial box.

The Ganoid scutes of the sturgeon are so far dominated by the huge chondrocranium, that, by courtesy, they may be called frontal, parietal, opercular, and the like; of course, such scutes are not the accurate homologues of the bones so named in the Teleostei, which, at the most, can only correspond to the inner layer of the scute of such a fish as the sturgeon.

The sturgeons, as a group, cannot be said to lie directly between any one family of the Selachians and any one family of the Bony Ganoids, yet, on the whole, that is their position; the Bony Ganoids, on the whole, approach the Teleostei, especially such forms as *Lepidosteus* and *Amia*, which have lost their "spiracle," and in other things are less than typical, as Ganoids.

Larval sturgeons are, in appearance, miniature sharks; for a few weeks they have a similar mouth, and their lips and throat are beset with true teeth, that are moulted before calcification has fairly set in. Their first gills are very long and exposed, but not nearly so long, or for such a time uncovered, as in the embryos of sharks and skates.

III. "On the Estimation of the Amylolytic and Proteolytic Activity of Pancreatic Extracts." By WILLIAM ROBERTS, M.D., F.R.S., Physician to the Manchester Royal Infirmary and Professor of Clinical Medicine in Owens College. Received April 23, 1881.

The degree of activity possessed by preparations of the soluble ferments cannot be ascertained by direct weighing and measuring. The agents to which they owe their power have in no case been obtained in a state of isolation and purity. These agents are known to be indissolubly united with some form of albuminoid matter, and we are constrained to speak of them as if they really were albuminoid bodies. But their mode of action suggests an affinity with the imponderable forces, and points to the conclusion that the relation which these

agents bear to their organic substratum is analogous, or at least comparable, to the relation subsisting between a mass of protoplasm and the vital endowments with which it stands possessed.

The activity of preparations of the soluble ferments can only be gauged by their capacity for work. But inasmuch as there is in them no power of growth and multiplication, the amount of energy with which they are endowed is strictly limited, so that when the capacity for work existing in a given liquid or solid preparation of one of these ferments has been ascertained, and has been put into due expression, the amount of energy in a certain quantity of the preparation can be counted in grams or cubic centimetres like that of any other chemical agent.

The term *ferment* has, up to this time, been applied in common to two groups of agents, which, although nearly allied both in their origin and in their mode of action, belong to essentially distinct categories. The organised or formed ferments, of which yeast is the type, are independent organisms with powers of growth and reproduction; and the transformations which constitute their special characteristics as ferments are inseparably associated with the nutritive operations of these organisms. The ferment-power cannot be separated from the ferment-organism by any method of filtration, nor by any solvent. The soluble ferments, on the other hand, pass freely into solution in water—their action is dissociated from the life of the gland-cells which produced them—and they are wholly devoid of the power of growth and reproduction.

Kühne has proposed to distinguish the group of soluble ferments by the name of “enzym.” I would suggest the desirability of adopting this term into English, with a slight change of orthography, as “enzymes,” and also of coining from this root the cognate words which are requisite for clear and concise description. The action of an enzyme may be designated *enzymosis*, and the nature of the action may be spoken of as *enzymic*. In the present paper I shall venture to employ these terms in the sense here indicated.

The pancreas is known to be the source of two ferments or enzymes, of capital importance in the digestion of food, namely, an amylolytic enzyme, *pancreatic diastase*, and a proteolytic enzyme, *trypsin*. It is also known that the pancreas takes an important share in the digestion of fats, but it is doubtful whether its power in this respect is due to an enzyme or to an agency of a different character. The present paper concerns itself solely with the amylolytic and the proteolytic functions of the pancreas.

*Estimation of the Amylolytic Activity of Pancreatic Extracts—
Diastasiometry.*

Probably the most accurate mode of estimating the activity of a

diastasic solution is to ascertain the amount of sugar produced when a given quantity of the solution is made to act on a given volume of a standard starch mucilage, for a fixed time and at a fixed temperature. This method has already been recognised by Mr. H. T. Brown and Mr. J. Heron in their paper on the transformation of starch by malt infusions.* Kjeldahl has developed this method to a further point, and has used it to measure the comparative activity of malt infusions and of saliva.†

In the method about to be described, a simpler and speedier proceeding was employed, and the results were so brought out as to indicate absolute as well as comparative values. In principle the method consists in ascertaining the quantity of starch mucilage of known strength which can be transformed, by a unit measure of a diastasic solution, to the point at which it ceases to give a colour reaction with iodine, in a unit of time, and at a fixed temperature.

When starch mucilage is treated with extract of pancreas, or with any other diastasic solution, the mixture progressively loses its property of giving a colour reaction with iodine. First the blue reaction of unaltered starch passes away, then the brown and yellow reactions of dextrine successively disappear. It is not difficult to fix, with a fair amount of accuracy, the vanishing point of this reaction. This point may, for our present purpose, be called the *achromic point*.

The extract of pancreas employed in these observations was prepared by digesting fresh pancreas, freed from fat and chopped up, in four times its weight of dilute alcohol, containing 25 per cent. of rectified spirit (sp. gr. 0·838). The digestion was continued for four or five days, with occasional agitation. The mixture was then filtered through paper. Filtration is much facilitated by the addition to the solvent of 0·02 per cent. of acetic acid (containing 28 per cent. dry acetic acid).

The *standard starch mucilage* was made from potato starch. Owing to the large size of its granules, potato starch is easily obtained in a state of great purity, by repeated levigation with water, and afterwards drying the product at 40° C.‡ The mucilage was made of the strength of 1 per cent., and in the following manner: 5 grms. of starch were well stirred up into a thin mud with 30 cub. centims. of water, and then poured in a slender stream into 470 cub. centims. of briskly boiling water. The mixture was stirred and allowed to boil for a few seconds. Thus prepared the mucilage is perfectly smooth and uniform, and is so diffuent that it can be measured out like an ordinary liquid. This is known in the present paper as the *standard*

* "Journal of the Chemical Society," September, 1879.

† "Compte Rendu des Travaux du Laboratoire de Carlsberg," 1879, p. 129.

‡ The so-called pure starch of the shops is worthless for the purposes of diastasi-metry. A supply of pure potato starch may be obtained from Mottershead & Co., Chemists, Manchester.

starch mucilage. It should be used fresh, for it is apt to change in a few days, and to lose its opalescent appearance, and slight mucilaginous consistency. When thus changed it is found to contain sugar. So long as it maintains its slight opalescence and slight mucilaginous character it is fit for use.

The *iodine solution* used in the testing was composed of 1 part of the *Liquor Iodi* of the British Pharmacopœia, diluted with 200 parts of water.

In determining the data on which is based the method of diastasi-metry here proposed, it was necessary to ascertain the mutual relations in regard to the amyolytic process of three factors, namely, the *quantity* of pancreatic extract set in action, the *time* required to reach the achromic point, and the *temperature* at which the enzymosis was carried on.

Quantity and Time.—The amount of amyolytic work done by a given sample of pancreatic extract is strictly proportional to the quantity of it set in action—in other words, the amount of the standard starch mucilage which can be changed to the achromic point in a given time and at a given temperature, varies directly as the quantity of the extract employed. This law of proportionality may probably be regarded as fundamentally applicable to the action of all enzymes, which, having no power of growth or multiplication, conform in this respect to the common law which governs the action of ordinary chemical agents. The rule is, however, liable to interference if the products of the enzymosis accumulate in the solution to such a degree as to hamper the action. In the conditions observed in the following experiments this interference did not arise. The starch mucilage operated on was exceedingly dilute, and consequently the sugar and dextrines produced in the transformation never accumulated to such a degree as to check the enzymosis.

In the action of all enzymes the element of time is an essentially important factor. An enzyme liberates its energy gradually, in successive portions, and it takes a comparatively long time to exhaust itself completely. I found that pancreatic diastase, in the presence of excess of starch mucilage, took not less than forty-eight hours to completely exhaust itself at the temperature of 40° C.—(“Lumleian Lectures” for 1880, “On the Digestive Ferments,” p. 38).

The fundamental rule which governs the mutual relations of quantity and time in the action of an enzyme is that of *inverse proportion*. That is to say, that double the quantity of an enzyme will do a given amount of work in half the time, and that half the quantity will require double the time. This rule, however, is apparently controlled by another rule, namely, that *an enzyme liberates its energy at a progressively retarded rate*. If we conceive an enzyme as a body in a state of tension, charged with a certain amount of dormant energy,

we can further conceive that in action it will discharge this energy gradually, and also at a rate which is continually diminishing. Such a conception will, I think, enable us to understand some features in the action of diastase and trypsin which are otherwise difficult to explain.

In regard to the action of pancreatic extract on starch mucilage, the rule of inverse proportion between quantity and time was found to hold good within considerable limits, as the following experiments show:—

Table I.

Experiments showing the inverse proportion between quantity and time in the action of pancreatic extract on starch mucilage. The quantity of the standard mucilage acted on in each experiment was 10 cub. centims. diluted with water up to 100 cub. centims. Temperature 15° C. The "calculated" time in the third column was obtained by taking the middle observation to each set as a standard of comparison.

	Quantity of pancreatic extract employed.	Time in which the achromic point was reached.	
		Found.	Calculated.
I....	0·02 cub. centim.	34 minutes	36 minutes
	0·04 "	18 "	18 "
	0·08 "	9 "	9 "
	0·10 "	7 "	7 $\frac{1}{5}$ "
	0·20 "	3 "	3 $\frac{1}{2}$ "
II....	0·4 "	4 $\frac{3}{4}$ "	5 "
	0·2 "	10 "	10 "
	0·05 "	40 "	40 "

In both sets of observations the inverse time-rate is seen to come out true with almost mathematical accuracy.

When, however, a relatively small quantity of pancreatic extract was employed, and the time required to reach the achromic point was, in consequence, considerably lengthened, it was found that the advent of the achromic point was postponed beyond the term indicated by the rule. If the period occupied in reaching the achromic point fell within the compass of an hour, and the temperature was low, as in the observation just recorded, the inverse time-rate came out true, but when the period of action extended to several hours and the temperature stood higher, the departure from the rule was undoubted. The annexed table gives the results of an experiment made with a view of testing this point.

Table II.

Experiments showing the postponement of the achromic point when the action is protracted. The quantity of standard mucilage acted on in each experiment was 10 cub. centims. diluted with water up to 100 cub. centims. Temperature 40° C. The "calculated" time in the third column was obtained by taking the first observation, which was several times repeated, as a standard of comparison.

Quantity of pancreatic extract employed.	Time in which the achromic point was reached.	
	Found.	Calculated.
0·05 cub. centim.	10 minutes.	—
0·005 "	115 "	100 minutes.
0·004 "	140 "	125 "
0·002 "	300 "	250 "
0·0005 "	1,380 "	1,000 "

It need scarcely be said that when the enzymosis is very slow it is not possible to fix the vanishing point of the colour reaction with the same precision as when the action is more rapid and the change more abrupt. Notwithstanding this source of error, I think the conclusion indicated by the experiments may be relied on. The postponement of the achromic point shown in the table may be explained, as has been suggested, on the assumption that the enzyme liberates its energy at a continually retarded rate. In the case of trypsin, we shall see evidence of a precisely parallel phenomenon.

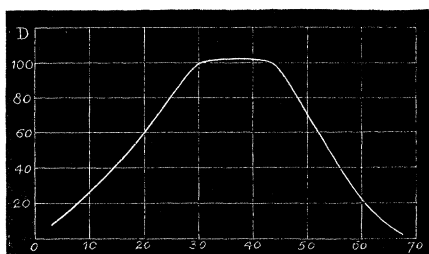
Temperature.—The action of pancreatic diastase on starch mucilage was found to increase in energy (or speed) from zero up to 30° C. From this point to 45° the rate of action continued steady, showing a range or platform of indifferent temperature extending from 30° to 45°. Above 45° the action became less and less energetic, and finally ceased between 65° and 70°. The following table exhibits the results obtained at various temperatures between 5° and 70°.

Table III.

Showing the effects of temperature on the action of pancreatic diastase; the amount of the standard mucilage acted on in each experiment was 10 cub. centims. diluted with water up to 100 cub. centims. The quantity of pancreatic extract employed in each experiment was 0·1 cub. centim.

Temperature.	Achromic point reached in
3—5° C.	36 minutes.
10	18 „
15	12 „
20	8 „
25	6 „
30	5 „
40	5 „
45	5 „
50	7 „
55	10 „
60	40 „
65	Very slow action.
70	No action.

These results, thrown into the form of a curve, are shown in the subjoined diagram. The ordinates indicate the diastasic value, or D, as calculated by a method to be presently explained; the abscissæ represent the temperatures.



Curve illustrating the effect of temperature on the action of pancreatic diastase.

Mode of proceeding.—In testing the activity of a sample of pancreatic extract, it was found on the whole more convenient to operate on a fixed quantity of the standard mucilage, and to vary the quantity of extract added to it, than to proceed contrariwise. The bulk of liquid operated on was thus kept constant. The ordinary proceeding was as follows: 10 cub. centims. of the standard mucilage were mixed in a beaker with 90 cub. centims. of water. The mixture was then warmed to 40° C., or at least to some point well within the range of indifferent temperature extending from 30° to 45°. This was done in order to eliminate the disturbing influence of temperature. The next step was to add the determined quantity of the extract to be tested to the diluted mucilage, and to note the exact time. Then, at short intervals, a drop of the enzymosing liquid was placed on a white slab, or plate, with a drop of the iodine solution. The time and result

of each testing was noted. When the achromic point was reached the time was marked, and the interval from the commencement of the experiment was computed. If at the end of three minutes the mixture still gave the blue reaction of unaltered starch, a new experiment was made, using two, three, or four times the quantity of extract. If, on the other hand, the achromic point was reached in less than two minutes, a new experiment was made, using a smaller quantity of the extract. Two or three experiments generally sufficed to determine the quantity of extract required to bring the achromic point within a period ranging from two to ten minutes. A final control experiment enabled the operator to fix the achromic point somewhere between four and six minutes. The accuracy of the method depends chiefly on the sharpness and precision with which the occurrence of the achromic point can be determined. If it occur earlier than two minutes, the transition is too rapid for exact observation and record. On the other hand, if it occur later than fifteen or twenty minutes the transition is too gradual for precise limitation. The most satisfactory results are obtained when the achromic point falls between four and six minutes.

The following example will serve as an illustration of the way in which the experiments were carried out, noted, and expressed:—

Table IV.

10 cub. centims. standard starch mucilage + 90 cub. centims. water
+ 0.1 cub. centim. pancreatic extract—at 40° C.

Time.	Reaction with iodine.
10.30 A.M.	Commencement of experiment.
10.31 „	Blue.
10.32 „	Violet.
10.33 „	Brown.
10.34 „	Yellowish-brown.
10.35 „	Pale yellow.
10.36 „	No reaction—achromic point.

6 minutes.

Achromic point reached in 6 minutes.

The result of the experiment was expressed in the first instance as follows: 0.1 cub. centim. pancreatic extract + 10 cub. centims. standard mucilage = 6 minutes at 40° C.

From this somewhat incongruous expression it is however easy to extract by a simple formula a correct and convenient expression for the diastasic value of any amyolytic solution.

Mode of Calculating and Expressing the Diastasic Value.

The principle of the method consists, as already stated, in ascertaining

the amount of starch mucilage of known strength which can be transformed by a unit measure of the diastasic solution to the point at which it ceases to give a colour reaction with iodine, in a unit of time and at a given temperature.

In reducing this principle to a definite formula it was necessary to choose arbitrarily a unit of measure and a unit of time. The unit of measure fixed on was 1 cub. centim., and the unit of time 5 minutes. These selections seemed, on the whole, the best adapted for furnishing a convenient scale. On these bases the formula took the following form: the diastasic value of any solution—or *D*—is expressed by the number of cubic centimetres of the standard starch mucilage which can be transformed to the achromic point by 1 cub. centim. of the solution to be tested in a period of five minutes at a given temperature.

In the process of testing the quantity of the standard mucilage was made constant, namely 10 cub. centims., and the quantity of pancreatic extract and the time were made variable. In order to get the value of *D* the results must be so transformed as to make the quantity of extract and the time constant, and the quantity of the standard mucilage variable. This is accomplished by increasing or reducing the quantity of pancreatic extract employed to 1 cub. centim., and increasing or diminishing the standard mucilage in the same proportion. The product thus obtained is again increased or reduced in the same proportion as is requisite to increase or reduce the time found to five minutes. Taking the example above given, the value of *D* is obtained by the following formula: Let *p* signify the quantity of pancreatic extract employed, and *m* the number of minutes required to reach the achromic point, then:—

$$\frac{10}{p} \times \frac{5}{m} = D,$$

and in the above example—

$$D = \frac{10}{0.1} \times \frac{5}{6} = 83 \text{ at } 40^{\circ} \text{ C.}$$

The value of *D*, as already explained, signifies the number of cubic centimetres of the standard starch mucilage which can be changed to the achromic point by 1 cub. centim. of the diastasic solution in five minutes at a given temperature. As the standard mucilage contains 1 per cent. of dry starch, the value of *D* divided by 100 gives us the same value in terms of dry starch, and the result of the above experiment may be read as follows:—

$$D = 83 = 0.83 \text{ grm. of dry starch.}$$

This method of diastasiometry is equally applicable to saliva and malt-diastase. It may also be applied to the estimation of the

diastasic agent which is present in urine, and presumably to all diastasic solutions. In the case of solid preparations containing diastase like malt or glandular tissue—a solution in known proportions must first be prepared; and from the ascertained activity of such solution the proportionate activity of the solid substance can be easily calculated. I may here mention some of the results which this method has already yielded.

Pancreatic Tissue.—The pancreatic tissue of the pig (obtained from animals killed for the market in the fasting state) yielded an extract which, when made on the large scale, possessed a mean diastasic value of 100. This extract is sent out by Mr. Benger, of the firm of Mottershead and Co., Chemists, Manchester, under the name of “Liquor Pancreaticus,” and is made in the proportion of one part of pancreatic tissue to four of solvent (water containing 25 per cent. rectified spirit). This value indicates that 1 grm. of the moist pancreas of the pig is capable of transforming 4 grms. of dry starch to the point at which it no longer gives a colour reaction with iodine, in five minutes, at a temperature of 40° C.

The pancreatic tissue of the ox and sheep yielded an extract (made in the same proportions) which was of far inferior activity. The ox extract had a diastasic value of about 11 and that of the sheep of about 12. These numbers indicate that in point of diastasic activity the pancreas of the pig has ten times the value of the pancreas of the ox and sheep. This extraordinary difference is probably linked with the diversity of their food. The pig is fed largely upon potatoes and meal, which are rich in starch; the ox and sheep, on the other hand, feed on grass, which is poor in starch. We shall presently find that there is no such difference in regard to tryptic activity in the pancreas of these animals.

Human Saliva.—Filtered saliva was found to have a diastasic value varying from 10 to 17 at 40° C. Its action was influenced by temperature exactly in the same manner as that of pancreatic extract. It increased in energy up to about 30° C., continued steady from this point to about 45°, and then declined, being finally extinguished between 65° and 70°.

Malt Diastase.—Infusions of malt made in the proportion of one part of crushed malt to four parts of water, exhibited a diastasic value of 4 to 5 at 40° C. But malt diastase did not attain its maximum activity at this temperature. It continued to increase in energy up to about 60° C., when it showed a diastasic value of 10. Above 60° the action diminished in energy, but did not come to a full stop until the temperature approached 80° C.

Human Urine.—Several specimens of healthy urine were tested by this method. They showed a diastasic value varying from 0·03 to 0·13 at 40° C. The effect of temperature thereon was not examined.

Estimation of the Proteolytic Activity of Pancreatic Extracts—Trypsimetry.

The writer had found in previous inquiries that when milk is subjected to digestion with pancreatic extract, a striking change takes place in it at an early stage of the process. The milk acquires the property of curdling when boiled. The onset of this reaction occurs at an earlier or at a later period according to the activity of the extract and the quantity of it employed; and it is possible to fix the time of its advent with considerable accuracy—sufficient accuracy to serve as the basis of a method of measuring the proteolytic activity of pancreatic extracts.

The reaction in question depends on the production, as a first step in the pancreatic digestion of casein, of a modified form of that body which I have named *metacasein*. This substance resembles casein in being curdled by acetic acid in the cold; but it differs from casein in being also curdled by simple boiling. These two reactions together distinguish metacasein from other proteid bodies.

The property of curdling when boiled, which may be called the *metacasein reaction*, continues observable in milk undergoing tryptic digestion until near the termination of the process; it then disappears somewhat abruptly, and the milk, when boiled, remains fluid just as it did at first.

We may, therefore, speak of the *onset point* of the metacasein reaction, and of the *vanishing point* of the metacasein reaction. These two points mark respectively the initial and the terminal limits of the principal phase in the digestion of milk by pancreatic extract.

Before the onset point of the reaction—that is, distinct and undoubted curdling—is actually reached, its approach is indicated by an appearance of soiling of the sides of the test-tube in which the milk has been boiled. This appearance is due to incipient coagulation, which presently develops into pronounced curdling, and is a useful sign in testing to indicate the coming on of the metacasein reaction.

The following typical experiment may serve to give the reader a clear notion of the succession of events—so far as they concern us here—which occur when milk is submitted to digestion with pancreatic extract.

Table V.

4 cub. centims. pancreatic extract added to 50 cub. centims. milk diluted with water to 100 cub. centims. Temp. 18° C.

Time.	Reaction on boiling.
2 minutes ..	No change.
3 „ ..	Slight soiling of the sides of the test-tube.
4 „ ..	More soiling.
5 „ ..	Distinct curdling—onset point of the metacasein reaction.

Time.	Reaction on boiling.
6 minutes ..	More pronounced curdling.
10 to 80 ,, ..	Pronounced curdling.
90 ,, ..	Diminished curdling.
95 ,, ..	Slight curdling.
100 ,, ..	No change; vanishing point of the metacasein reaction.

The length of time during which the successive steps of the transformation may continue observable depends on the energy of the action; and this, in its turn, depends on the activity of the preparation and the quantity of it added to the milk; it is also greatly influenced by temperature. By using an excess of an active pancreatic extract, and with a favourable temperature, all the steps of the process may be crowded almost into an instant of time; with converse conditions the action may linger on for many hours.

Although milk is a secretion of somewhat variable composition, the oscillations which it exhibits, when it is the product of a dairy, and is not intentionally adulterated, do not materially vitiate it for the purposes of a test fluid such as is here required. The milk delivered at my house presented very little variation. It had a density of 1030—seldom varying more than a degree from this point—and the results obtained with the milk of different days showed a remarkable uniformity. Milk from different dairies, and at different seasons of the year, would no doubt present greater irregularities. Milk should, however, be used fresh, for if it have become slightly acid, as it is apt to do in keeping, the results obtained are untrustworthy.

If milk be diluted with water the occurrence of the metacasein reaction is postponed; and the degree of postponement varies with the degree of dilution. For example, if 50 cub. centims of pure milk are changed to the onset point of the metacasein reaction in three minutes, the same quantity of milk diluted with an equal volume of water will take six minutes to reach the same point—other conditions being equal. There are, however, several advantages in using diluted milk instead of pure milk as the experimental fluid. The inequalities of the milk are thereby minimised. The “strike” of the reaction is more sharply defined, and the required quantity of pancreatic extract can be included in the water of dilution. This last is an important advantage, because if the extract to be tested is feeble, a considerable quantity of it requires to be added, and this, if pure milk were employed, would seriously alter its degree of dilution, and thereby vitiate the results. In the following experiments, milk diluted with an equal bulk of water was invariably employed; and if the quantity of pancreatic extract to be added exceeded 3 cub. centims. for every 50 cub. centims. of milk, this was always included in the water of dilution.

In principle the method of trypsimetry here proposed consists in

ascertaining how many cubic centimetres of milk can be changed to the onset point of the metacasein reaction, in 5 minutes, by 1 cub. centim. of the extract to be tested, at a given temperature.

In settling the data on which the method is based, it was necessary, as in the case of diastase, to determine the relations to tryptic action of *quantity, time, and temperature.*

Quantity and Time.—The rule of inverse relations between quantity and time which was found to be valid within a wide range in the case of diastase and starch, is only reliable in the case of trypsin and milk within narrow limits. When the time of action exceeds 8 or 10 minutes the advent of the metacasein reaction is postponed beyond the term indicated by the rule of inverse proportion, and this postponement increases as the time of action is lengthened. The following two sets of observations may be taken as samples of the results obtained by experiment.

Table VI.

Showing the postponement of the metacasein reaction.

The quantity of milk acted on in each experiment was 50 cub. centims. diluted with water up to 100 cub. centims. The "calculated" time in the third column was obtained by taking the first observation in each set as the standard of comparison.

Quantity of pancreatic extract added.	Onset point of the metacasein reaction.	
	Found.	Calculated.
Set I.—Temperature 40° C.		
1·0 cub. centim.	3 minutes.	—
0·8 "	4 "	3¾ minutes.
0·6 "	5 "	5 "
0·4 "	9 "	7½ "
0·2 "	30 "	15 "
Set II.—Temperature 16° C.		
4·0 cub. centims.	6 minutes.	—
2·0 "	16 "	12 minutes.
1·0 "	39 "	24 "
0·5 "	105 "	48 "
0·25 "	280 "	96 "

When the vanishing point of the metacasein reaction was taken as the point of comparison, the results approximated more nearly to the requirements of the rule of inverse proportion, especially at low temperatures; but still the evidence pointed in the same direction, and indicated that trypsin, like diastase, exhausts itself in action at a pro-

gressively retarded rate. From the numerous experiments which were performed with a view of elucidating this point, I arrived at the conclusion that when the onset point of the metacasein reaction fell between 3 and 6 minutes the inverse time-rate gave a reliable basis of calculation, but not beyond these limits.

Temperature.—Tryptic enzymosis is exceedingly sensitive to temperature. The action of trypsin on milk increases in energy from zero to 60° C. Above this point there is a rapid fall, and the action is finally arrested between 75° and 80° C. There is not, as with diastase, any range or platform of indifferent temperature. The following table exhibits the degrees of activity from 10° to 80°. In order to obtain the utmost uniformity of results, the quantities of pancreatic extract employed were so adjusted as to bring the incidence of the metacasein reaction within a period ranging from 4 to 6 minutes.

Table VII.

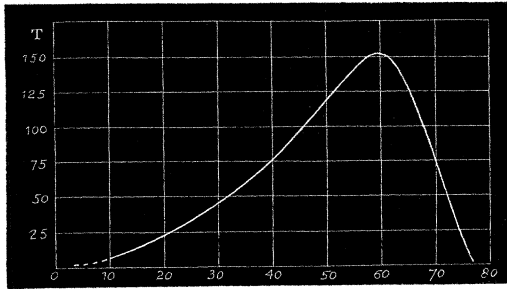
Showing the effects of temperature on tryptic enzymosis.

The quantity of milk employed in each experiment was 50 cub. centims. diluted with water up to 100 cub. centims. In the fourth column the degree of tryptic activity, or T, is calculated by a method to be presently explained.

Temperature.	Quantity of pancreatic extract employed.	Onset point of the metacasein reaction.	Tryptic value— or T.
10° C.	6·0 cub. centims.	5 minutes.	8
15	4·0 ”	5 ”	12
20	3·0 ”	4 ”	21
30	1·0 ”	5½ ”	45
40	0·6 ”	5½ ”	76
50	0·4 ”	5¼ ”	119
60	0·3 ”	5½ ”	150
65	0·4 ”	5 ”	125
70	0·8 ”	4 ”	78
75	2·0 ”	6 ”	21
80	4·0 ”	No action.	0

In the subjoined diagram these results are thrown into the form of a curve. The ordinates indicate the degrees of tryptic activity (or T), and the abscissæ indicate the temperatures.

In another series of experiments the effect of temperature was gauged by the length of time required to reach the onset or the vanishing point of the metacasein reaction when constant quantities of pancreatic extract were used. The results obtained in this series are tabulated in Table VIII. In the first set the onset point of the reaction was taken as the index of tryptic activity; in the second set



Curve illustrating the effect of temperature on the tryptic digestion of milk.

the vanishing point of the reaction was employed for the same purpose. The results brought out by these experiments correspond pretty closely with those given in Table VII.

Table VIII.

Showing the effect of temperature by the length of time required to reach the metacasein reaction, when constant quantities of pancreatic extract are used.

I Set. 0.4 cub. centim. pancreatic extract with 100 cub. centims. diluted milk.		II Set. 4 cub. centims. of pancreatic extract with 100 cub. centims. diluted milk.	
Temperature.	Onset point of the metacasein reaction.	Temperature.	Vanishing point of the metacasein reaction.
2 to 5° C.	312 minutes.	—	—
10	168 "	10° C.	180 minutes.
15	120 "	—	—
20	70 "	20	75 "
30	25 "	30	26 "
40	12 "	40	12 "
50	6 "	50	6 "
60	4 "	60	4 "
65	6 "		
70	} action suspended, but resumed on cooling.		

An examination of the table shows how very nearly the results correspond, whether the onset point or the vanishing point of the metacasein reaction be taken as a measure of tryptic activity. This correspondence substantiates the conclusion that the onset point of the reaction furnishes a trustworthy index of the activity of tryptic digestion. The proportionate quantity of pancreatic extract added to the milk in the experiments recorded in Set II of Table VIII was ten

times as great as in those recorded in Set I; and it is seen that, by using these proportions, the vanishing points and the onset points fell out in nearly the same times in both sets of experiments.

Mode of Proceeding.—In testing the tryptic activity of a sample of pancreatic extract, the following procedure was adopted:—50 cub. centims. of fresh milk were diluted with 50 cub. centims. of water, less the quantity of extract intended to be added. The diluted milk was then warmed to 40° C., and maintained exactly at that temperature until the close of the experiment. The intended quantity of the pancreatic extract, say 1 cub. centim., was then added, and the time exactly noted. At the end of each minute a portion of the digesting milk was withdrawn, and boiled for a few seconds in a test-tube, inclining the test-tube to one side after the boiling in order to observe the effect. The result was at once noted down. As soon as distinct curdling occurred on boiling, the experiment was considered finished; the time was recorded, and the number of minutes which had elapsed from the commencement of the experiment were reckoned. The result came out in the following form:—

1 cub. centim. panc. extract + 50 milk = 4 minutes at 40° C.

If no signs of incipient curdling (soiling of the sides of the test-tube) occurred within 3 minutes, a new experiment was made, using, two, three, or four times as much pancreatic extract. If, on the other hand, distinct curdling occurred in 2 minutes, or less, a fresh experiment was made, using half or quarter the quantity of extract. Three or four such experiments usually sufficed to enable the operator to fix the onset point of the reaction somewhere between 4 and 6 minutes.

Mode of Calculating and Expressing the Tryptic Value.—The object of the experiment was to ascertain how many cubic centimetres of milk can be changed to the onset point of the metacasein reaction by 1 cub. centim. of extract in a period of 5 minutes, at the temperature of 40° C. The tryptic value, or T, was calculated from the first expression of the results of an experiment in exactly the same way as for diastase. If p be made to signify the quantity of pancreatic extract added to the milk, and m the number of minutes which were required to reach the onset point of the metacasein reaction, then the value of T was obtained by the following formula:—

$$\frac{50}{p} \times \frac{5}{m} = T,$$

and taking the experiment above given the value of T came out as follows:—

$$T = \frac{50}{1} \times \frac{5}{4} = 62.5 \text{ at } 40^\circ \text{ C.}$$

In judging the practical value of this method of trypsimetry, one must have regard to the inherent difficulty of estimating the activity

of preparations of the proteolytic enzymes. I venture to think that we have in this method a means of estimating the activity of trypsin preparations which is superior in ease and precision to any we possess for the evaluation of pepsin preparations. What may be the limits of error arising from inequalities in the composition of milk I am unable to say, but with the same specimen of milk the limits of error do not certainly exceed 6 to 8 per cent.

The tryptic value of pancreatic extract from the pig, made on the large scale, was found to range from 40 to 70 at 40° C. The pancreatic tissue of the ox and sheep yielded an extract which possessed about the same tryptic activity as that of the pig. Extracts prepared from single glands presented very considerable variations both in regard to their diastasic and their tryptic activity. The following table shows the enzymic values of twelve samples of pancreatic extract prepared with single glands from four pigs, four oxen, and four sheep, killed for the market.

Table IX.

(All the observations were made at 40° C.)

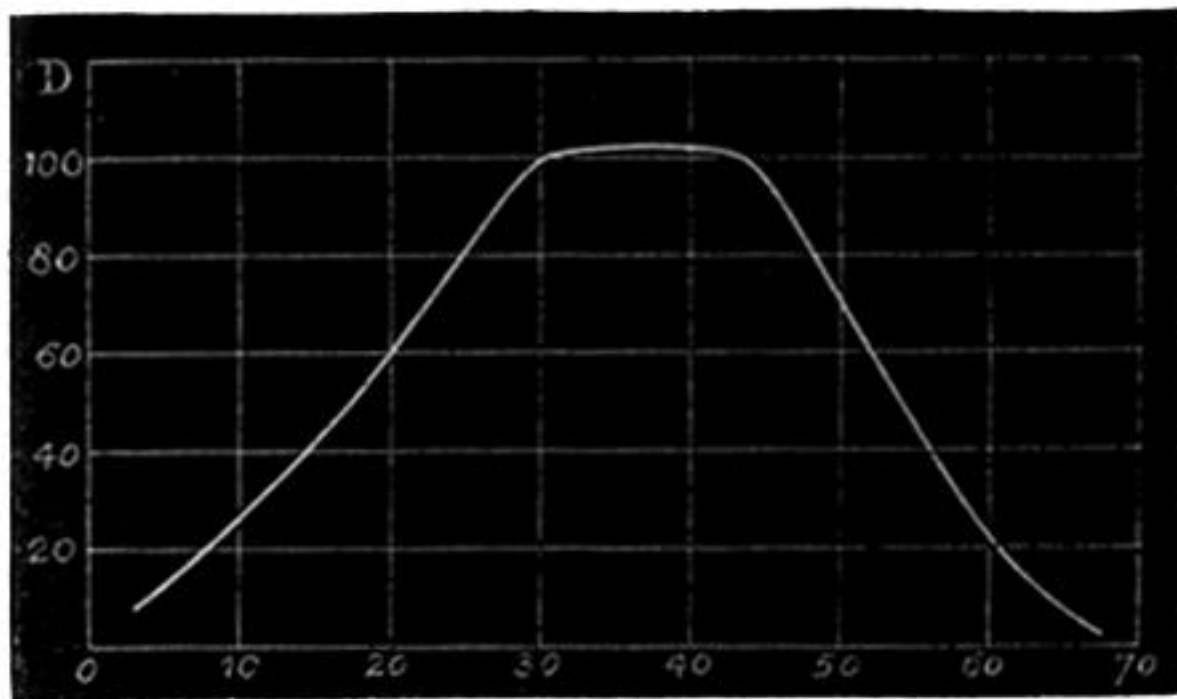
(D stands for diastasic value, and T for tryptic value.)

Fig.	Ox.	Sheep.
No. 1 { D = 166 T = 64	No. 5 { D = 8 T = 64	No. 9 { D = 5 T = 125
No. 2 { D = 100 T = 83	No. 6 { D = 10 T = 50	No. 10 { D = 12 T = 83
No. 3 { D = 100 T = 72	No. 7 { D = 9 T = 42	No. 11 { D = 14 T = 64
No. 4 { D = 100 T = 64	No. 8 { D = 13 T = 83	No. 12 { D = 4 T = 28

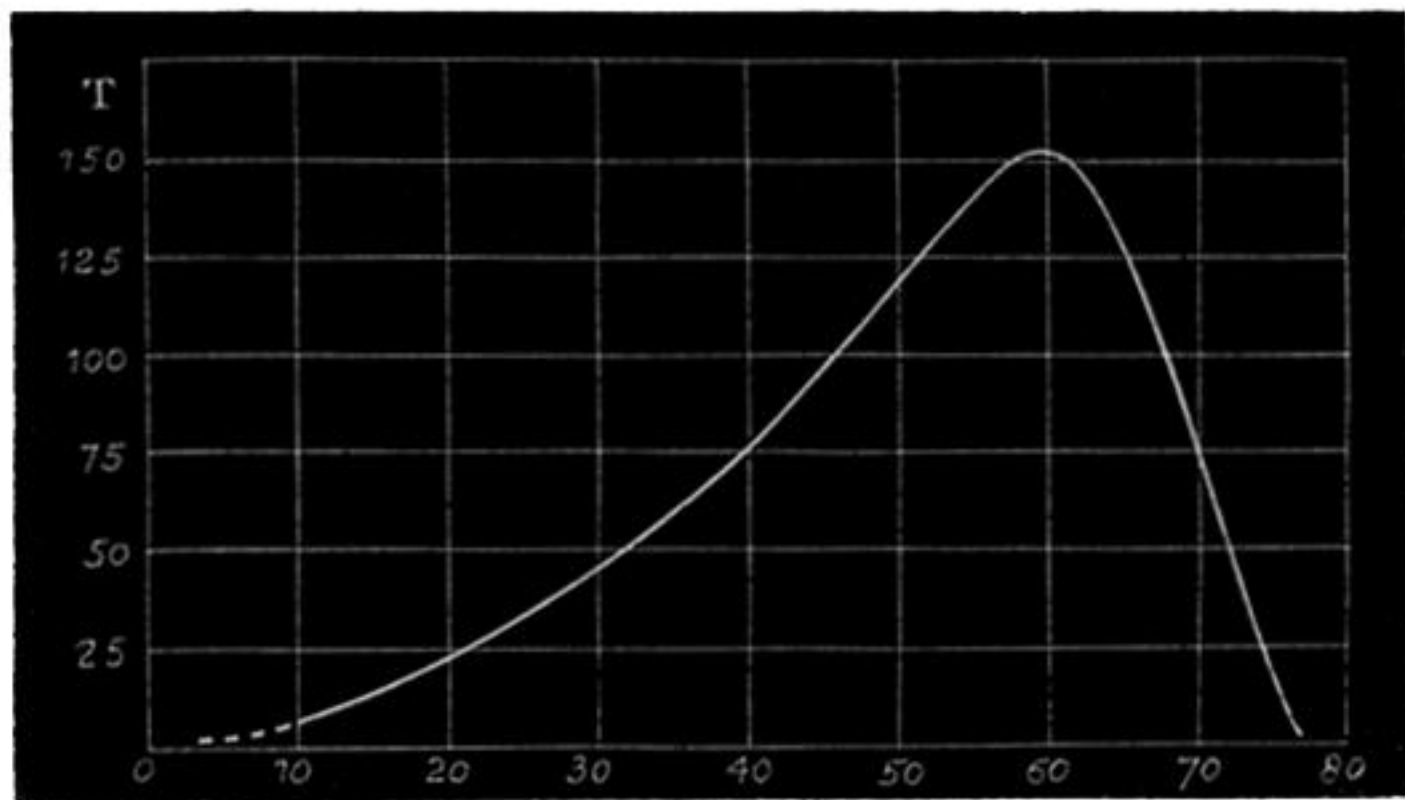
It may be observed that the oscillations in the two enzymic values bear no mutual relations to one another.

The most appropriate standard of temperature for the valuation of tryptic activity, is 40° C., because this corresponds very nearly with the temperature at which trypsin operates in the normal digestion of warm-blooded animals. But it is more convenient to perform the testing at, or near, the ordinary temperature of the room (say, at 20°) inasmuch as in the latter case, it is much less troublesome to maintain a continuously uniform temperature than at 40°.

I have, therefore, taken some pains to ascertain the exact relation between the value of T at 40° and at 20° respectively, and have found that at 40° the value of T is very nearly three and a half times as great as at 20°. If, therefore, the testing be performed at 20°, the resulting value of T multiplied by 3.5, will give with sufficient accuracy the value of T at 40°.



Curve illustrating the effect of temperature on the action of pancreatic diastase.



Curve illustrating the effect of temperature on the tryptic digestion of milk.