CLXI. THE DISTRIBUTION OF XANTHINE OXIDASE I.

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IN a previous paper by Morgan, Stewart and Hopkins [1922] it was shown that "xanthine oxidase" acts as a typical dehydrase in the sense of Wieland. Tissue preparations which contain the enzyme oxidise the bases hypoxanthine and xanthine to uric acid anaerobically in the presence of methylene blue, the dye being reduced to its colourless leuco-compound.

The use of this anaerobic technique, compared with the method of adding known amounts of the bases to a tissue preparation and estimating at the end of the experiment the amount of unchanged xanthine, or hypoxanthine, is so simple that it becomes comparatively easy to test for the presence or absence of the catalyst in the case of any given tissue. As only small amounts of material are required it has the additional advantage that the organs of the smaller animals can be investigated and a large number of organs can be easily compared at the same time.

It was the purpose of the present research to extend the very limited number of animals in which the distribution of the enzyme has been hitherto studied, and its results yield further evidence to show that the xanthine oxidase, like the other enzymes concerned with the breakdown of purines, has a peculiar and limited distribution among the organs of a given species, while the localities in which it is found vary considerably from species to species.

RÉSUMÉ OF THE METHOD.

The tissue preparations are conveniently made by Bach's method. The organs from which the preparations are to be made are first freed from obvious fat and connective tissue. The larger organs are then passed through a mincer and sampled. The stomach and intestines are thoroughly washed out with water before using.

The smaller, and from 5 to 10 g. of the larger, organs are ground up with 5 or 10 volumes of 2 % sodium fluoride solution in a mortar, using a little washed sand, and squeezed through strong, fine muslin. It is better to pound the tissue well with the sand before adding a portion of the measured sodium fluoride solution and grinding. After squeezing this through muslin, the débris from the muslin is returned to the mortar and again ground, using the rest of the sodium fluoride solution; after squeezing this through the muslin only connective tissue should remain.

It is important that the preparations should not be filtered through filter paper, for Dixon and Thurlow [1924, 1] have shown that the enzyme is readily absorbed by paper.

The preparations, which are essentially cell suspensions, are transferred to Erlenmeyer flasks, lightly plugged with cotton wool, and allowed to stand with an occasional shake at room temperature, or better at 25°, for 24 to 48 hours, when their original power to reduce methylene blue will be greatly diminished.

From 2 cc. to 5 cc. of the preparations, which should be well shaken and if necessary again squeezed through muslin to obtain a good sample, are then measured into two vacuum tubes similar to those used by Thunberg and described by Hopkins and Dixon [1922]. To one is added a measured quantity of hypoxanthine solution, and to the control the equivalent amount of solvent. To each tube is added the same quantity of methylene blue solution. The tubes are well evacuated, placed in a thermostat with a glass side at 37°, and the reduction time noted.

In the following experiments 0.2 cc. to 0.5 cc. of a freshly prepared $M/100$ solution of hypoxanthine was used, dissolved either in $N/50$ sodium carbonate solution, or in 0.2N NaOH solution and buffered to p_H 7.8 with 0.2M KH_2PO_4 . The solution in phosphate buffer was used with tissue preparations which are only slightly buffered and in which, upon adding the $N/50$ sodium carbonate solution, the p_H was above 8.0. Dixon and Thurlow [1924, 2] have shown that the reaction velocity is unaffected by p_H between 5.5 and 9.0.

If it is found that the reducing power of any of the preparations, without added hypoxanthine, is too high to give a blank of 2 to 3 hours using 0.5 cc. and from 3 to 6 hours using 1.0 cc. of $M/1000$ methylene blue solution, they can either be left to stand longer before use, or diluted with an equal volume of 2 % sodium fluoride solution. Otherwise the amount of methylene blue can be increased.

When increasing the strength of the methylene blue the following data should be remembered. 1 cc. of $M/1000$ methylene blue solution is equivalent to 11.2 mm.³ of oxygen at N.T.P.; 1 cc. of $M/100$ hypoxanthine solution (0.00136 g.) oxidised to uric acid is equivalent to 224 mm.³ of oxygen at N.T.P., which is equivalent to 20 cc. of $M/1000$ methylene blue solution.

The animals were either killed at the slaughter-house and the organs brought fresh to the laboratory, or they were chloroformed in the laboratory and bled from the jugular vein.

It is important that the animals should have no fresh milk for several hours before killing or a positive result may be obtained for the stomach and small intestine.

With the exception of the smaller organs, five volumes of 2 % sodium fluoride were used throughout, and the preparations allowed to stand 24 to 48 hours. In the case of the earthworm and caterpillar, which darken upon standing, 2 cc. of methylene blue were used and the preparations tested after standing for 2 hours without shaking with air.

It would be satisfactory if the evidence could be given *in extenso* but this would call for much space. The full data are therefore presented for three animals only, but when they are recorded in abbreviated form the evidence obtained was, in all cases, equally unequivocal. The result of adding hypoxanthine to a preparation is, in the case of certain organs, to reduce the time necessary for the reduction of a standard quantity of methylene blue so greatly that hours may become minutes. In the case of other organs there is, within the limits of observational error, no effect at all. Only in the rarest of instances is the effect upon the reduction time small enough to be equivocal. The evidence for the presence of the enzyme in any given case is therefore always beyond doubt, and so, with certain qualifications to be discussed later, is the evidence that in another case it is absent. That the actual reduction time in the controls (tissue preparations without added purine base) varies somewhat widely is due, in the first place, to variations in the general reducing powers of different tissues, and, in the second, to variations in the degree to which reducing systems in a given tissue were exhausted before its preparations were employed in an experiment. The latter factor was intentionally varied by varying the time of standing before employment. Examination of the data will show that the definiteness of the results was not affected by any such variation in the controls. All comparisons were of course simultaneous and the only difference between one system and the other was in each case the presence or absence of added hypoxanthine.

MAMMALS.

Table I. *The Dog.*

R.T. = Time for complete reduction.

A plus sign in the minutes column indicates that the observation ceased before reduction was complete, no difference being then observable between the two tubes compared.

Tissue preparation		R.T. control	R.T. hypoxanthine	Xanthine oxidase
Liver	Dog 1	2 hrs. 40 mins.	2 hrs. 40 mins.	—
	" 2	4 " 0 "	4 " 7 "	—
	" 3	2 " 10 "	2 " 8 "	—
	" 4	4 " +	4 " +	—
Kidney	" 1	2 " 40 "	2 " 45 "	—
	" 2	2 " 52 "	2 " 52 "	—
Spleen	" 1	4 " 10 "	0 " 45 "	+
	" 2	2 " 51 "	0 " 24½ "	+
Heart	" 1	3 " 25 "	3 " 25 "	—
	" 2	2 " 43 "	2 " 46 "	—
Muscle	" 1	3 " 2 "	3 " 8 "	—
	" 2	5 " +	5 " +	—
Lung	" 1	6 " 7 "	0 " 33 "	+
	" 2	5 " 4 "	0 " 15 "	+
Pancreas	" 1	5 " 10 "	5 " 16 "	—
	" 2	5 " 20 "	5 " 14 "	—
Thymus	" 1	5 " 6 "	5 " 6 "	—
	" 2	5 " 40 "	5 " 36 "	—
Stomach	" 1	3 " 9 "	3 " 12 "	—
	" 2	6 " 14 "	6 " 23 "	—
Small intestine	" 1	3 " 50 "	0 " 21½ "	+
	" 2	3 " 52 "	0 " 13 "	+
Large intestine	" 1	7 " +	7 " +	—
	" 2	3 " 35 "	3 " 35 "	—

For comparison of the methylene blue technique with the method of adding the purine bases to a tissue preparation and estimating at the end of the experiment the amount of unchanged xanthine or hypoxanthine and uric acid, I have used the organs of the dog. Using the latter method, xanthine oxidase has been shown by Jones and Austrian [1906] and Schittenhelm [1909] to be present in dog's spleen, intestine and lung, but absent from the liver, kidney, pancreas and muscle. It will be seen from Table I that the methylene blue technique very sharply confirms these authors.

Table II. *The Cat.*

Tissue preparation		R.T. control	R.T. hypoxanthine	Xanthine oxidase
Liver	Cat 1	2 hrs. 10 mins.	0 hrs. 17 mins.	+
	" 2	1 " 2 "	0 " 9 "	+
	" 3	1 " 16 "	0 " 14½ "	+
	" 4	2 " + "	0 " 18 "	+
Kidney	" 1	2 " 13 "	2 " 13 "	-
	" 2	3 " 5 "	3 " 9 "	-
	" 3	3 " 10 "	3 " 7 "	-
Spleen	" 1	9 " + "	9 " + "	-
	" 2	7 " + "	7 " + "	-
Heart	" 1	7 " + "	7 " + "	-
	" 2	3 " 18 "	3 " 21 "	-
Muscle	" 1	3 " 5 "	3 " 5 "	-
	" 2	8 " + "	8 " + "	-
Lung	" 1	1 " 42 "	0 " 12½ "	+
	" 2	8 " + "	0 " 50 "	+
	" 3	5 " 25 "	0 " 58 "	+
Pancreas	" 1	8 " + "	1 " 22 "	+
	" 2	8 " + "	1 " 44 "	+
	" 3	6 " 10 "	0 " 50 "	+
Thymus	" 1	10 " + "	10 " + "	-
	" 2	8 " + "	8 " + "	-
Stomach	" 1	6 " 40 "	6 " 40 "	-
	" 2	7 " + "	7 " + "	-
Small intestine	" 1	7 " + "	0 " 33 "	+
	" 2	8 " + "	0 " 24 "	+
Large intestine	" 1	4 " 17 "	4 " 10 "	-
	" 2	7 " + "	7 " + "	-

Table III. *The Sheep.*

Tissue preparation		R.T. control	R.T. hypoxanthine	Xanthine oxidase
Liver	Sheep 1	1 hrs. 50 mins.	0 hrs. 7 mins.	+
	" 2	1 " 33 "	0 " 6 "	+
Kidney	" 1	2 " 28 "	2 " 30 "	-
	" 2	2 " 58 "	2 " 58 "	-
Spleen	" 1	2 " 40 "	2 " 40 "	-
	" 2	5 " 5 "	5 " 17 "	-
Heart	" 1	3 " 10 "	3 " 6 "	-
	" 2	3 " 50 "	3 " 55 "	-
Muscle	" 1	2 " 15 "	2 " 15 "	-
	" 2	4 " 39 "	4 " 35 "	-
Lung	" 1	3 " 8 "	3 " 13 "	-
	" 2	9 " + "	9 " + "	-
Pancreas	" 1	2 " 33 "	2 " 33 "	-
	" 2	3 " + "	3 " + "	-
Thymus	" 1	4 " 31 "	4 " 31 "	-
	" 2	3 " 53 "	3 " 49 "	-
Stomach	" 1	10 " + "	10 " + "	-
	" 2	6 " + "	6 " + "	-
Small intestine	" 1	10 " + "	10 " + "	-
	" 2	8 " + "	8 " + "	-
Large intestine	" 1	5 " 46 "	5 " 50 "	-
	" 2	7 " + "	7 " + "	-

The figures in the preceding experimental data have been selected from a number of duplicates. A difference of a few minutes in the reduction times of a control tube and of one to which hypoxanthine has been added, in the case of a preparation which takes several hours to reduce, can be disregarded as falling within the limits of experimental error. In the following shortened presentation of the data obtained from other animals, the figure after the name of an organ indicates the number of individual animals studied in each case.

The Goat.

Liver (2) +. Kidney (2) -. Spleen (2) -. Heart (2) -. Muscle (2) -. Lung (2) -. Pancreas (2) -. Thymus (2) -. Stomach (2) -. Small intestine (2) -. Large intestine (2) -.

The Rabbit.

Liver (2) +*. Kidney (2) -. Spleen (2) -. Heart (2) -. Muscle (2) -. Lung (2) -. Pancreas (2) -. Thymus (2) -. Stomach (2) -. Small intestine (2) +. Large intestine (2) -.

* Xanthine oxidase has been shown to be present in rabbit's liver by Jones and Austrian [1906]. I have confirmed the work of these authors.

The Hedgehog.

Liver (3) -. Kidney (2) -. Spleen (2) -. Heart (2) -. Muscle (2) -. Lung (2) -. Pancreas (2) -. Thymus (2) -. Stomach (2) -. Small intestine (2) +. Large intestine (2) -. Mammary gland (active) (2) +.

BIRDS.

The Pigeon.

Liver (3) -. Kidney (2) +. Heart (2) -. Muscle (2) -. Lung (2) -. Pancreas (3) +. Gizzard (2) -. Small intestine (2) -. Large intestine (2) -.

The Fowl.

Liver (5) +. Kidney (2) +. Spleen (2) -. Heart (2) -. Muscle (2) -. Lung (2) -. Pancreas (5) -. Gizzard (2) -. Small intestine (2) -. Large intestine (2) -.

The remarkable contrast in respect of their content of the enzyme displayed by the liver of closely related species is so noteworthy as to justify the experimental data being given in full (Table IV).

Table IV.

Liver preparation		R.T. control	R.T. hypoxanthine	Xanthine oxidase
Dog	1	2 hrs. 40 mins.	2 hrs. 40 mins.	-
"	2	4 " 0 "	4 " 7 "	-
"	3	2 " 10 "	2 " 8 "	-
"	4	4 " + "	4 " + "	-
"	5	2 " 6 "	2 " 6 "	-
"	6	2 " 18 "	2 " 22 "	-
Cat	1	2 " 10 "	0 " 17 "	+
"	2	1 " 2 "	0 " 9 "	+
"	3	1 " 16 "	0 " 14½ "	+
"	4	2 " + "	0 " 18 "	+
Fowl	1	1 " 15 "	0 " 22 "	+
"	2	2 " 3 "	0 " 29 "	+
"	3	1 " 18 "	0 " 2½ "	+
"	4	2 " 6 "	0 " 4 "	+
"	5	1 " 25 "	0 " 8 "	+
Pigeon	1	4 " 27 "	4 " 27 "	-
"	2	3 " 9 "	3 " 6 "	-
"	3	3 " 7 "	3 " 13 "	-

INVERTEBRATES.

The Lobster.

Liver (2) -. Green gland (3) -. Heart (2) -. Muscle (2) -. Gills (2) -. Stomach (2) -. Testes and seminal vesicles (2) -. Blood (2) -.

The Earthworm.

Whole worms (4) -. Pharynx -. Crop and gizzard -. Intestine (upper) -. Intestine (lower) -. Nephridia -. Sections containing hearts -. Muscle -. Seminal vesicles -*.

* Combined organs from a large number of earthworms.

I also found the enzyme to be absent from the oyster, and present in the caterpillar and pupae of *Pieris brassicae*. It is also present in rat carcinoma.

In Table V most of the available data obtained by others, as well as by myself, are collected together, so that the varying distribution of the enzyme can be clearly seen. The references to the previous literature of the data given are contained in Abderhalden [1911], Oppenheimer [1911], Wells [1910], Caldwell and Wells [1914] and Morgan, Stewart and Hopkins [1922]. The data furnished by other observers are marked by an asterisk.

Table V.

	Man	Monkey	Ox	Horse	Sheep	Goat	Pig	Dog	Cat	Rabbit	Rat	Opossum	Hedgehog	Pigeon	Fowl
Liver	+	+	+	?	+	+	+	+	+	+	+	+	-	+	+
Kidney	-	-	+	?	-	-	-	-	-	-	+	+	-	+	+
Spleen	-	-	+	+	-	-	-	+	-	-	+	+	-	+	-
Heart	?	-	?	?	-	-	?	-	-	-	?	?	-	-	-
Muscle	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-
Lung	-	-	+	?	-	-	-	+	+	-	+	-	-	-	-
Pancreas	-	-	-	?	-	-	-	-	+	-	?	?	-	+	-
Thymus	?	?	-	?	-	-	?	-	-	-	?	?	-	?	?
Stomach	?	-	?	?	-	-	-	-	-	-	?	?	-	-	-
Small intestine	+	-	+	-	-	-	-	+	+	+	?	-	+	-	-
Large intestine	?	-	?	?	-	-	?	-	-	-	?	?	-	-	-
Mammary gland (active)	?	?	?	?	?	?	?	?	?	?	?	?	+	.	.

DISCUSSION.

As was earlier indicated, and as will be seen on examination of the results given in the tables, wherever the presence of the enzyme in a tissue has been claimed in this research, the evidence yielded by the use of the anaerobic technique has been unequivocal. Whenever the absence of the enzyme has been claimed the effect of such addition has been, in practically every case, completely negative.

The negative results, however, call perhaps for some comment. It is well known, of course, that throughout a certain range in the relative concentration of any enzyme and its substrate an increase in the latter may fail to increase the velocity of reaction, the enzyme surface being saturated from the beginning. In a given tissue extract or cell suspension, such as those used in this research,

the breakdown of nuclein materials may be in progress, and if the production of xanthine and hypoxanthine from their precursors is faster than their destruction, they will accumulate while the preparation is allowed to stand. The oxidase, if present in relatively low concentration, might then be saturated with its substrate when the experiment is begun, so that no decrease in reduction time would follow upon the addition of purine base. When, however, tissue preparations are allowed to stand long enough before use to render their reducing power for methylene blue so small as to be negligible, the addition of base is still without effect in cases where the absence of the enzyme has been claimed, but always enormously decreases the reduction time in the case of tissues which undoubtedly contain the enzyme. The latter show that the catalyst is not readily destroyed under the conditions of experiment. It would seem, therefore, that the considerations put forward in the last paragraph have no important bearing on the results. When, with the technique employed, the addition of purine base has no observable effect upon reduction time, the enzyme cannot be present in a concentration which would be physiologically significant.

The suggestion may arise that, when a tissue fails to oxidise xanthine or hypoxanthine, this is due not to the absence of the active catalyst, but to the presence of an inhibitory factor. The results of such experiments as the following speak against such a view. The spleen of the ox is rich in the enzyme, whereas, if the general evidence be accepted, that of the pig contains none. A preparation was made of each, and both preparations were allowed to stand until their reducing power for methylene blue was very small, and their uptake of oxygen in Barcroft's apparatus negligible. The ox spleen preparation was then mixed (*a*) with an equal volume of the pig spleen preparation, and (*b*) with an equal volume of sodium fluoride solution. All conditions being the same each mixture exhibited the same oxidative power, anaerobically and aerobically. Each by itself showed a reduction time of about 4 hours (concentrations being as in the majority of the experiments reported). In each case the addition of the standard amount of hypoxanthine shortened the reduction time to 12 minutes. In the Barcroft apparatus neither mixture showed any significant uptake of oxygen. When to 2 cc. of each, 1 cc. of *M*/100 hypoxanthine solution was added, the oxygen uptake was identical (286 mm.³ in 11 hours) and the velocity curves were nearly superposable. The oxidation was clearly due to the ox spleen alone, whilst the pig spleen was without effect. Such results may not wholly prove the absence of an inhibitory factor from the latter; but they make against a hypothesis which is in itself not very likely.

A survey of the results given in Table V leads to the following conclusions.

The enzyme is present in the livers of ten out of the twelve mammals examined. It is however absent from that organ in the case of the dog and the hedgehog. The difference in this respect between the carnivores, the dog and the cat respectively, is so definite and noteworthy that full details are

given in Table V. Its absence from the liver of the dog had been shown previously by Jones and Austrian [1906] and my own observations upon six individuals fully confirm the circumstance. That it is present in cat's liver is equally sure.

It is absent from the kidney of all the mammals examined¹ save in the case of the opossum. In this its appearance would seem to be variable [Caldwell and Wells, 1914], a circumstance not without interest in the case of a marsupial, since its presence in the kidney of birds—to judge from the two species examined—seems definite.

In the case of mammalian organs other than the liver and kidney its distribution is highly variable from species to species. In man and in monkeys it may be confined to the liver though all the organs have not been examined. In three ungulates (sheep, goat and pig) it appears to be wholly confined to that organ. In the ox on the other hand it is certainly also present in the spleen, lung, and small intestine, and the cat shows a similar distribution save for its absence from the spleen and presence in the pancreas. The hedgehog offers a case in which under ordinary circumstances it would seem to be only in the small intestine. I found however that the enzyme is present in highly efficient concentration in the active mammary gland of the last-named animal, and also in that of the guinea-pig. Since the presence of the enzyme is characteristic of milk a further investigation of the mammary gland, passive and active, would be of interest. Taking the rat as an example of a small animal with a high basal metabolism it is to be noted that the enzyme is here probably widely distributed; the negative results of Rohde and Jones [1909] have been explained by Dixon and Thurlow [1924, 1]. It is certainly present in the liver, kidney and spleen, and also in the lung; other organs in this animal have unfortunately not been examined. Save the fact that it is generally present in the liver and absent from the kidney of mammals, and to the former at least of these statements there is in the dog a noteworthy exception, no general statement is possible.

No genetic relationships in the distribution are obvious, though, to decide concerning the possibility of these, many more representative species would have to be studied.

It would not be surprising to find where a factor of purine metabolism is concerned that birds differ from mammals. To judge from the two cases I have studied such a difference is found in the unquestionable presence of the xanthine oxidase in the avian kidney. Here again, however, we find a remarkable difference between species; the enzyme is certainly present in the liver of the fowl, and with equal certainty absent from that of the pigeon.

It would be interesting to know whether in the metabolism of species with

¹ The kidney of the ox is exceptional in not yielding the clear-cut results (positive or negative) given by other tissues. My observations suggest that it contains the enzyme in very low concentration. Schittenhelm [1905] has stated that it is present, but if his figures are consulted they will be found to show at most a doubtful activity.

a limited distribution of the enzyme there is a greater escape of xanthine and hypoxanthine than in others which contain it more widely distributed. The only data available on this point are contained in the comparative studies of Hunter and Givens [1914] and of Hunter, Givens and Guion [1914]. Even with these, however, it is impossible to generalise. It may be noted, however, that in the case of the three ungulates, sheep, goat and pig (the pig was studied by Schittenhelm [1910]), the excretion of purine bases is relatively high, and in these animals the oxidase is present in the liver only. In the ox, on the other hand, the excretion of bases is low and the oxidase is widely distributed among its organs.

The identity of xanthine oxidase with the Schardinger enzyme.

Dixon and Thurlow [1924, 2] working with enzyme preparations from milk have put forward certain evidence, which they, however, do not feel is sufficient to justify a positive statement, to suggest that the xanthine oxidase is identical with the Schardinger enzyme.

Although a large number of data obtained by myself seem to lend support to this view they cannot perhaps, be put forward as valid evidence for assuming the identity of the two enzymes. Yet, it is very significant that in all the tissues which I have investigated, where the presence of xanthine oxidase was demonstrated, replacement of the purine base by acetaldehyde in neutral solution, always induced a fairly rapid reduction of the methylene blue. Where, on the other hand, the xanthine oxidase was proved absent, the addition of aldehyde was in many cases without effect. It should be noted, however, that, in some of the tissues which do not contain xanthine oxidase a definite decrease in the reduction time may follow upon the addition of acetaldehyde, when the control is from 6 to 10 hours; this is most marked in sheep's lung. This reduction must be further investigated before any conclusions can be drawn, but, as Dixon [1926] points out, "it is quite certain that there are other enzymes which will oxidise aldehydes, but which are altogether without action upon hypoxanthine."

SUMMARY.

1. The organs of the following mammals, the sheep, goat, rabbit, cat and hedgehog, and those of the domestic pigeon and fowl, have been investigated for the presence of xanthine oxidase. The tissues of the following invertebrates were also studied, the lobster, oyster, the caterpillar and pupae of *Pieris brassicae*.

2. Evidence regarding the identity of xanthine oxidase with the Schardinger enzyme is put forward.

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REFERENCES.

- Abderhalden (1911). *Biochem. Handlexikon*, 5, 645.
Caldwell and Wells (1914). *J. Biol. Chem.* 19, 279.
Dixon (1926). *Biochem. J.* 20, 703.
Dixon and Thurlow (1924, 1). *Biochem. J.* 18, 971.
——— (1924, 2). *Biochem. J.* 18, 976.
Hopkins and Dixon (1922). *J. Biol. Chem.* 54, 527.
Hunter and Givens (1914). *J. Biol. Chem.* 18, 403.
Hunter, Givens and Guion (1914). *J. Biol. Chem.* 18, 387.
Jones and Austrian (1906). *Z. physiol. Chem.* 48, 110.
Morgan, Stewart and Hopkins (1922). *Proc. Roy. Soc. Lond. B.* 94, 109.
Oppenheimer (1911). *Handbuch Biochem.* 4 (1), 506.
Rohde and Jones (1909). *J. Biol. Chem.* 7, 237.
Schittenhelm (1905). *Z. physiol. Chem.* 45, 121.
——— (1909). *Zentr. ges. Physiol. Path. Stoffw.* Nr 21.
——— (1910). *Z. physiol. Chem.* 66, 53.
Wells (1910). *J. Biol. Chem.* 7, 171.