CLIII. CARBOHYDRATE METABOLISM IN BIRDS.

III. THE EFFECTS OF REST AND EXERCISE UPON THE LACTIC ACID CONTENT OF THE ORGANS OF NORMAL AND RICE-FED PIGEONS.

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Kinnersley and Peters [1929, 1930] have demonstrated that there is an increase in the lactic acid content of the brain of the pigeon in polyneuritis (induced by feeding on polished rice) and that the increase is particularly marked in the mid- and hind-brain. They were inclined to interpret their results as an indication that a local defect in the metabolism of this lower part of the brain was responsible for the symptoms of polyneuritis, but it seemed to be of interest to investigate the lactic acid contents of other organs of the body in order to determine whether the phenomenon they observed was in fact a part of a more general disturbance. With this end in view a technique was developed for the use of iodoacetic acid as a "fixative" for lactic acid in the organs of the pigeon, in the hope that it might prove possible to obtain satisfactory estimates of the lactic acid contents of the pectoral muscle, the heart and the liver of the same bird. A preliminary account of this technique has already been published [Fisher, 1931].

In the course of an attempt to obtain standard conditions in the birds used for estimations it was discovered accidentally that the lactic acid contents of organs of polyneuritic pigeons depended to a considerable extent on the conditions of the experiment. This finding has been expanded, and in consequence the results, as presented, are not strictly complementary to those of Kinnersley and Peters. But they serve the original purpose in that they demonstrate that there is a definite general defect in lactic acid metabolism in the polyneuritic pigeon, in addition to the local defect described by Kinnersley and Peters.

Methods.

Lactic acid fixation. The use of iodoacetic acid as a fixative is based on Lundsgaard's [1930] original observation that lactic acid is apparently not formed in the stimulated gastrocnemius of a frog previously poisoned with the drug. The applicability of his assumption to normal pigeon muscle was

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tested by the injection, intraperitoneally and intravenously, into a small series of pigeons of small amounts of sodium iodoacetate. Pectoral muscle from these pigeons was minced and incubated in $M/15$ phosphate buffer, p_H 7, at 37° for 2 hours. In three instances out of four the muscle showed a decrease in lactic acid content, and in the fourth a slight increase was observed. The decrease was not significant unless lactic acid were added to the muscle before incubation.

In view of the possible quantitative importance of the disappearance of lactic acid observed, experiments were made upon the effect of the addition of iodoacetic acid in buffered solution to finely minced unpoisoned muscle. This muscle contained initially from 400 to 550 mg. of lactic acid per 100 g. The rates of glycolysis observed in the presence of these high concentrations of lactic acid ranged from 120 to 610 mg. of lactic acid formed per 100 g. of tissue per hour, at 37°, when the muscle was suspended in buffer in a concentration of 1 g. of muscle to 5 cc. of buffer. But the range of rates of disappearance observed in the same system on the addition of iodoacetic acid was only from 40 to 70 mg./100 g. tissue. The results of these experiments are presented in Fig. 1. It should be noted that control experiments have

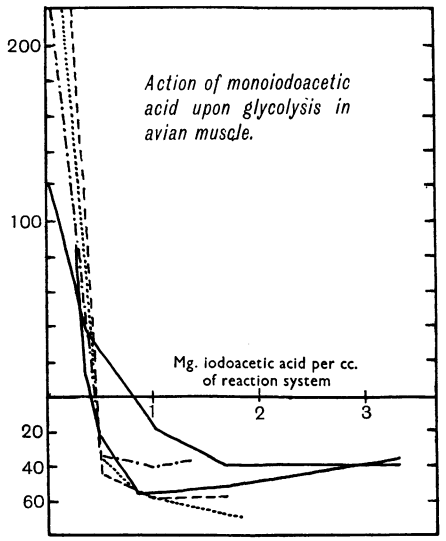

Fig. 1.

shown that no destruction of lactic acid occurs when lactic acid is incubated with iodoacetic acid in the absence of muscle tissue.

Fig. 1 indicates that, in a method of lactic acid estimation by fixation, the error which is produced by lactic acid destruction is likely to be very much less than the corresponding error introduced by glycolysis into the only other method available, *i.e.* rapid excision of tissues from a suitably anaesthetised animal, and immersion of the excised tissues in liquid air. But the figure

understates the case. Two rough experiments in which the iodoacetic acid content of a series of flasks was held constant, whilst the lactic acid content was varied by the addition of *dl*-lactic acid, showed that even at a concentration of 2·5 % total lactic acid (or 1·25 % *d*-lactic acid) the system causing the lactic acid disappearance was not saturated with respect to lactic acid. The rate of disappearance at this concentration was 100 % greater than that at a concentration of 0·5 % *d*-lactic acid. Since the concentration of lactic acid in the tissues taken for lactic acid estimation is in all probability about 0·01–0·02 %, judging by the figures obtained by Davenport and Davenport [1927] for guinea-pig and rat muscle, the rate of disappearance of lactic acid from the tissues under the conditions of *in vivo* fixation by iodoacetic acid should be too small to be of any account.

Since the data of Fig. 1 provide no guide to the best dosage for *in vivo* fixation of lactic acid by iodoacetic acid, the dose had to be found by trial and error. The only criteria available were the uniformity of the results and certain figures which had been obtained in a few preliminary experiments in which I had assisted Professor Peters. In these experiments the pigeons had been anaesthetised with "Liquid Dial," a proprietary non-volatile anaesthetic containing diallylbarbituric acid and urethane, and tissue samples had been removed as rapidly as possible and plunged into liquid air. The range of concentrations of lactic acid found is indicated in Table I. It is of interest to note the extremely high values for the hearts both of the normal and polyneuritic birds. The higher values were the more prevalent in both series, yet all the hearts were beating when they were excised, and it is doubtful if more than 3 secs. ever elapsed between excision and complete freezing. If these figures be compared with those in Table II it will be seen how extraordinarily rapid *post mortem* glycolysis must be in this tissue.

Table I. *Lactic acid content of pigeon tissues; liquid air method.*

Normal	Muscle: 22–35 mg./100 g. tissue
	Heart: 35–94
	Liver: 42–78
Polyneuritic	Muscle: 77–89
	Heart: 63–141
	Liver: 22–96

In all these experiments glycolysis must have occurred to some extent, so that any figure obtained by a successful iodoacetic acid method ought to fall below the lowest figure in the corresponding group in Table I.

Using this criterion it has been found that:

(1) "Dial" anaesthesia militates against success.

(2) Intravenous injection is unsatisfactory.

(3) Fixation is very unreliable in birds which die more than 12 minutes after the injection.

(4) Doses larger than 150 mg./100 g. body weight tend to cause stoppage of the heart before the drug has been adequately distributed.

The method finally adopted was to inject intraperitoneally a dose of 50 or 75 mg. of the drug per 100 g. body weight, the bird being unanaesthetised. The iodoacetic acid was dissolved in 5 % NaOH and made just alkaline to phenol red.

Of the total of 38 sets of estimations reported in this paper 19 were made using a dose of 50 mg./100 g., 18 were made using 75 mg., and one was made using a dose of 100 mg./100 g. of an impure commercial preparation. The higher dosage was used when it became apparent that the figures for the pectoral muscle obtained at the lower dosage were not satisfactory. However, as may be seen from Table II, the increase in dosage had little if any effect on

Table II. *Lactic acid concentrations in pigeon tissues: iodoacetic acid technique.*

1	2	3	4	5	6	7	8	9
		Dose of iodoacetic acid mg./100 g. body-weight	Lactic acid: mg./100 g.			Time to death in mins.	Length of exercise in mins.	Length of rest in mins.
Group	Exp.		Muscle	Heart	Liver			
Rested normals	1	50	6	6	8	4	None	25
	2	50	—	8	14	7	,,	37
	3	50	(39)	6	—	6	,,	37
	4	50	(24)	13	6	12	,,	130
	5	50	(71)	11	21	7	,,	31
	6	50	—	11	19	7	,,	80
	7	75	14	10	8	5	,,	52
	8	75	(25)	—	13	10	,,	45
	9	50	(139)	13	19	7	,,	75
Normals from cage	1	50	11	12	14	—	None	None
	2	50	13	16	15	—	,,	,,
	3	50	(136)	17	10	—	,,	,,
	4	50	12	18	—	4½	,,	,,
	5	50	(28)	13	9	9	,,	,,
	6	50	12	10	7	10	,,	,,
Exercised normal	1	50	15	14	24	12	8	None
	2	75	21	19	18	9	17	,,
	3	75	19	20	32	10	6	,,
	4	75	19	28	54	7	10	,,
Long rest avit.	1	50	—	11	24	7	None	309
	2	50	16	4	19	6	,,	409
	3	75	32	17	20	13	,,	336
Short rest avit.	1	50	41	24	37	6	None	96
	2	50	—	27	—	7	,,	42
	3	50	59	21	20	11	,,	28
	4	75	37	33	35	7	,,	45
	5	75	(133)	27	15	6	,,	26
	6	75	58	26	—	6	,,	150
Exercised avit.	1	75	68	83	70	10	6	None
	2	100	87	76	80	5	5	,,
	3	75	66	51	53	9	11	,,
	4	75	49	64	62	9	10	,,
Cured avit.	1	75	15	18	18	6	—	49
	2	75	13	24	23	6	—	67
Exercised cured avit.	1	75	26	27	37	12	9	None
	2	75	31	20	55	7	16	,,
	3	75	54	33	51	8	11	,,
	4	75	22	26	48	10	12	,,

the nature of the results. The general conclusion which may be drawn from the figures is that the method is eminently satisfactory as far as the heart is concerned, less satisfactory for the liver (though it is conceivable that the range of variation found in the liver corresponds to a true variation of the same magnitude), and of doubtful value for the pectoral muscle. It is interesting to note that the results indicate quite definitely that the drug affects the glycolytic systems of all these tissues.

Lactic acid determination. Samples of the tissues required (one sample from each pectoralis major muscle, the heart, and the greater part of the liver) were removed after the bird had died, pressed between filter-papers to remove adherent blood, and placed in tared 50 cc. centrifuge tubes, each of which contained 10 cc. of 10 % trichloroacetic acid. The tubes were weighed and the tissues were ground up with a glass rod. The tubes were allowed to stand for 45–60 minutes, and the fluid was poured off. The process was repeated three times, using water as the extraction fluid on the last two occasions, and the filtered extracts were collected in 100 cc. flasks. 10 cc. of 10 % $CuSO_4$ solution and 10 cc. of 5 % $Ca(OH)_2$ suspension were added to each flask, and the volume was made up to 100 cc. with washings from the filter.

After standing for an hour the extracts were filtered, and the first 10 cc. of filtrate were rejected. Estimations of the lactic acid content of 10 cc. portions of filtrate were made by the Friedemann, Cotonio and Shaffer [1927] method. *N*/200 iodine, freshly made up, was used for the final titrations, and Na_2HPO_4 was used to liberate the bound bisulphite, as recommended by Lehnartz [1928]. Duplicate titrations agreed as a rule to within 0·02 cc. As the net titration figure ranged from 0·20 cc. to 1·40 cc. the final figures are probably within 10 % of the true values. The method recovers 100–103 % of a standard zinc lactate solution of the order of concentration of the lactic acid in the extracts.

Preliminary treatment of the pigeons.

Polyneuritic pigeons. These birds were obtained by feeding on a polished rice diet and were used in the head-retraction stage. The precautions recommended by Kinnersley, Peters and Reader [1928] to ensure that the head-retraction was truly polyneuritic in origin were observed in each instance.

Cured polyneuritic pigeons. These were birds belonging to the polyneuritic group which had received 1–3 g. of marmite in water by the mouth from 12 to 24 hours before the injection of iodoacetic acid. In all instances the birds were free from any sign of polyneuritis when they were taken, and none of them developed any such sign during exercise.

Rest. The pigeon was kept at rest by wrapping it in a cloth and placing it in a dosing-box.

During the resting period the pigeon was kept in the dark [see, in this connection, Benedict and Riddle, 1929] and at the end of the resting period it was usually possible to take the pigeon out of the box without any move-

ment on its part, provided that a cloth were placed gently over its eyes before it was brought into the light, and it were grasped carefully with the cloth still obscuring its vision. The bird could be turned slowly on to its back and the injection made. It should be noted that the degree of rest is less in the polyneuritic birds than in the normal ones, since the head-retraction persists for some considerable time after they are placed in the dosing-box.

Exercise. It was found to be difficult to ensure that the extent of the exercise given to the birds should be comparable in all the classes (*i.e.* in normal, polyneuritic and cured birds). The normal and cured birds were exercised by being made to fly, either freely, by throwing them into the air, or else by holding them by the feet or tail and repeatedly drawing them downwards through the air. In these circumstances they beat their wings vigorously. The polyneuritic birds were exercised by allowing them free play for their convulsions and by tumbling them about on the ground. In this way normal and cured birds were probably exercised to the same degree, but the severity of the exercise of the polyneuritic birds was definitely less than that of the birds in the two other clases.

Results.

The lactic acid concentrations found in the pectoralis major muscle, the heart muscle and the liver tissue of eight groups of birds are presented in Table II. The figures in brackets in column 4 represent lactic acid concentrations which are in excess of those of the corresponding groups of Table I, *i.e.* results which are obviously not true values. Unfortunately it is impossible, on the data available, to apply the test afforded by Table I to all the groups.

In order that the significance of the variations in lactic acid concentration from group to group may be more readily appreciated, the figures for the lactic acid contents of the heart have been rearranged in Fig. 2 in a different

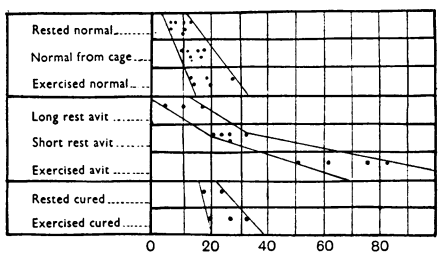

Fig. 2.

form. It has already been said that more reliance is to be placed on these results than on those for the pectoral muscle and liver, but it will be found that what is true for the heart lactic acid content is true also, though not so immediately apparent, for the muscle and liver.

Fig. 2 demonstrates that the effect of severe exercise on the lactic acid content of the heart of the normal pigeon is surprisingly small. Since all the evidence goes to show that in severe exercise lactic acid is liberated in considerable amount, this seems to mean that in the 7–12 minutes which elapse between the end of exercise and the death of the pigeon practically all the liberated lactic acid is converted into some other substance. In the polyneuritic pigeon, on the other hand, we see that the lactic acid content of the heart remains very high for at least 5–10 minutes after exercise, is still twice as high as the normal content in birds from the cage which have been rested for 30–150 minutes, and falls to the level of the resting normal bird only after 300 to 400 minutes' rest.

The only simple interpretation of these results, having regard to their regularity, is that, whereas the normal pigeon possesses a remarkably efficient mechanism for the removal from its tissues of the lactic acid which accumulates in exercise, this mechanism is crippled in great part in birds suffering from avian polyneuritis. This crippling might be an adventitious effect, arising out of the partial starvation which is a consequence of the anorexia accompanying the disorder. Evidence that this is not so is afforded by the observations upon cured birds. There is very little difference between the behaviour of the lactic acid content of the heart in exercise in these birds and in normal birds, whereas there is an immediately apparent difference between its behaviour in cured birds and polyneuritic birds.

As a further proof that what has been termed by some authors "inanition" has nothing to do with the behaviour of the lactic acid content of the heart, the weights of the cured birds at the beginning of rice-feeding, at the time of dosing with the yeast preparation, and at the time of injection with iodoacetic acid, are given for all instances in which accurate data are available. It will be seen (Table III) that the lactic acid content has reverted to normal before any significant rise in weight has occurred.

Table III. *Effect of curative extracts on the weights of polyneuritic pigeons.*

	Weight of bird in g.		
No. of bird in Table II	Before rice-feeding	When dosed	When used
Exercised cured 2	342	230	230
Exercised cured 3	317	192	194
Exercised cured 4	330	251	260
Rested cured 1	365	239	246
Rested cured 2	332	221	228

It seems clear that none of the changes of weight after dosing can be considered to be significant, and that the differences observed between polyneuritic and cured birds must be ascribed to the yeast preparation (in all but one instance marmite) which cured the polyneuritis.

Discussion.

These results are of interest in connection with those obtained by Inawashiro and Hayasaka [1928] and Hayasaka [1929], using Japanese subjects suffering from beriberi. They showed that the blood-lactic acid concentration remained at a high level after severe exercise in beriberi patients for longer than in normal men and cured beriberi patients. They also showed that this tendency to persistence at a high level of the lactic acid of the blood developed progressively in a young male Japanese subject in whom avitaminosis B was induced by feeding him for 200 days on a deficient diet.

Their results conform exactly with the hypothesis suggested to explain the findings reported in this paper.

One point on which some doubt might be felt is the validity of the assumption that while in the normal bird a considerable amount of lactic acid is formed in exercise most of it disappears in the first few minutes of rest. No evidence has been cited in support of this assumption, but Kinnersley and Peters [1930] found 14–34 mg./100 cc. of lactic acid in the blood of pigeons killed with the guillotine (these birds corresponded to the "normals from cage" of this paper), and 80–126 mg./100 cc. in the blood of birds that had been severely exercised (the exercise was in most instances even more severe than that given to the pigeons used in this investigation). These figures are very valuable, since a number of investigations have established that in the absence of substances which inhibit cellular respiration glycolysis does not occur in avian blood. Rüter [1923] established this for goose blood, and Bornstein and Ascher [1926] found the same thing to be true for hen's blood. Gulland and Peters [1930] extended the observations to pigeon's blood. The figures given above may therefore be taken to represent the true lactic acid contents of the blood at the time of death, and it may be considered as established that there is a high concentration of lactic acid in the blood of a normal pigeon at the conclusion of severe exercise. Presumably therefore there must be a concentration of the same order in the organs of the pigeon from which the lactic acid passes into the blood. The fact that Kinnersley and Peters's figures for the blood-lactic acid are on the whole higher than the corresponding figures for the heart (comparing their "normals" with my "normals from cage" and their "exercised" birds with my "exercised avit." birds) can be accounted for if one considers, in the first place, that the exercise they gave their birds was probably more severe, and, in the second place, that the lactic acid of the solid organ is probably not distributed throughout the organ, but is present for the most part in the free water of the tissue. This free water comprises about 77 % of frog's muscle, but no determinations have been made on pigeon tissues [Hill, 1930].

The figures for exercised birds in Table II may therefore safely be considered to demonstrate that a rapid disappearance of accumulated lactic acid takes place in the first 5–12 minutes after the cessation of exercise in a normal

bird, whereas there can be only a very slow disappearance of lactic acid in the polyneuritic bird.

The difficulty encountered by Kinnersley and Peters in relating high lactic acid concentration in the brain to the symptoms of polyneuritis is somewhat diminished on this view. They found that they were able, by exercise, to raise the lactic acid content of the lower parts of the brain in normal birds to the level which they habitually found in the brains of polyneuritic birds. But the normal birds showed no sign of polyneuritis in these circumstances. However, it now seems that the lactic acid content can only momentarily be kept at that height in the normal bird, whereas in the polyneuritic bird it will tend to remain practically stationary for some time. It may be that the persistence of the lactic acid causes an accumulation of some precursor which is responsible for the symptoms. This would fit in with Vogt-Møller's suggestion [1931] that the symptoms of polyneuritis may be due to the accumulation of methylglyoxal, although even Sjollema and Seekles's [1926] comprehensive tabulation of the toxic effects of methylglyoxal does not permit any definite conclusion to be drawn. Peters and Gavrilescu (see note p. 1406) have been unable to isolate methylglyoxal from the brains of polyneuritic pigeons.

SUMMARY.

A method is described for the fixation of the glycolytic systems in the organs of the pigeon by means of iodoacetic acid: the method is satisfactory for the heart, less satisfactory for the liver, and of doubtful value in most circumstances for the pectoral muscle.

Marked differences have been noted between the normal and the polyneuritic pigeon in the responses of the lactic acid contents of the heart, liver and muscle to exericse and rest. It is suggested that these differences signify that in the normal bird lactic acid formed in exercise is very rapidly removed from the tissues, but that in avian polyneuritis the mechanism of removal is very greatly crippled. This crippling appears to bear a close relation to the avitaminosis underlying avian polyneuritis.

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