SOME EFFECTS OF FASTING ON THE COMPOSITION OF THE BLOOD AND RESPIRATORY EXCHANGE IN FOWLS

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(With Six Text-figures.)

It has been shown that the blood sugar in the adult fowl, male or female, after falling during the first 2 days of fasting, rises on the 3rd or 4th day and then declines rapidly so that a well-defined peak is formed (Henry, Macdonald and Magee, 1933). This change was so constant in its occurrence that experiments were undertaken in the hope of throwing light on the immediate cause. As all the original glycogen stores are almost certainly exhausted after fasting for 3 or 4 days, the increase in blood sugar could only have come from protein or fat. A study was therefore made in fasting fowls of the uric acid and non-protein nitrogen contents of the blood, in the hope that, if any marked change in the metabolism of protein occurred, it would be reflected in alterations in the amounts of these present in the blood. In regard to fat the metabolites chosen were cholesterol and lecithin. The significance of these substances in fat metabolism is not very clear at present; but, as they can be readily and accurately estimated in small amounts of blood, they were preferred to blood fat as possible indicators of change in fat metabolism. The liver and muscle glycogen of fowls killed after different periods of fasting were also determined, as well as the effect of fasting on the respiratory exchange. Marked qualitative alterations in energy metabolism would, it was thought, be associated with corresponding changes in the R.Q. None of these lines of study threw any direct light on the problem we set out to solve; but the results obtained were considered to be of sufficient interest in themselves to merit publication.

THE EFFECT OF FASTING ON SOME BLOOD AND TISSUE CONSTITUENTS.

Fowls were fasted for periods up to 7 days in wire-bottomed cages, so that it was impossible for them to eat their excreta. Distilled water only was given. Samples of blood were drawn from the wing veins for all except the lecithin estimations, which required 5 c.c. of blood. This was obtained from fowls killed every 24 hours for the glycogen estimations. These fowls were killed by cutting the jugular vein, and then drawing the neck. Two samples of liver and of pectoral muscle were rapidly excised and their glycogen contents determined. Owing to the importance

of the 3rd and 4th days of fasting in regard to our objective, more fowls were killed at the end of 48, 72 and 96 hour periods than at the other times. The numbers of fowls used are shown in Table I. As the variations in blood sugar might have been due to changes in the non-glucose reducing substances, which are higher in the fowl than in the mammal, the true sugar, as well as the total reducing substances, were estimated. The analytical methods employed were: blood sugar (total reducing substance), Hagedorn and Jensen; true glucose, Blanco (1928); glycogen, Irving (1928); non-protein nitrogen, Folin and Svedberg (1930); uric acid, Benedict (1922); cholesterol, Leiboff (1924); lecithin, Stare and Elvehjem (1932). The fowls used were all pure-bred White Leghorn cocks.

RESULTS.

The results could not be stated as averages, because the fluctuations which occurred were not always constant in time and in direction; so that an increase, say in blood sugar at 96 hours, might be completely neutralised by a fall in another fowl at the same interval. Typical results only are therefore shown (Fig. 1). The curves of total reducing substances and true glucose, on the whole, ran parallel; so that the increases at 72 or 96 hours on the curve of total reducing substances can only have been caused by corresponding rises in the glucose of the blood. No constant change was shown in the N.P.N. values. The uric acid and cholesterol curves kept fairly constant until the 96th hour and then began to rise steadily until the end of the fast. This increase in the uric acid was doubtless brought about by increasing breakdown of tissues, in spite of which the N.P.N. was unaffected. Although the cause of this difference is far from clear, it is interesting because of the fact that a large quota of the total nitrogenous end-products in birds consists of uric acid. This result suggested that the sources of energy in the fowls before the 4th day were largely glycogen and fat. The cholesterol curve up to this point remained fairly steady, and therefore gave no indication as to whether or not increasing amounts of fat were being oxidised. But the fact, that the increase in the cholesterol content occurred at the same time as that of uric acid, would appear to be an indication of progressive tissue catabolism, since the alcohol is present in most cells and tissues.

The lecithin figures (Table I) unfortunately throw no light on the matter. They show that a dip occurred at 72 hours, but that at 96 hours the original level was reached, which remained almost unchanged until the end of the fast. The formation of soluble lipoids is believed to be the first stage in fat utilisation, and a decrease in blood lecithin probably indicates, according to Myers (1924), a fall in the rate of fat metabolism. This could only be due in fasting animals to exhaustion of the fat depots, the result of which would be a progressive diminution in fat metabolism and not merely a temporary fall as the above figures suggest. Ling (1931) found that both the lecithin and cholesterol contents of dog's blood were lowered by fasting, but he has quoted many results pointing in the opposite direction.

The glycogen data (Table I) were disappointing. Clearly no deduction can be made from those for muscle. The average liver glycogen values, however, suggest

that the percentage was higher at 72 and 96 hours than at any of the other intervals during the fast. Unfortunately, the range of values was rather wide—0·102–0·256

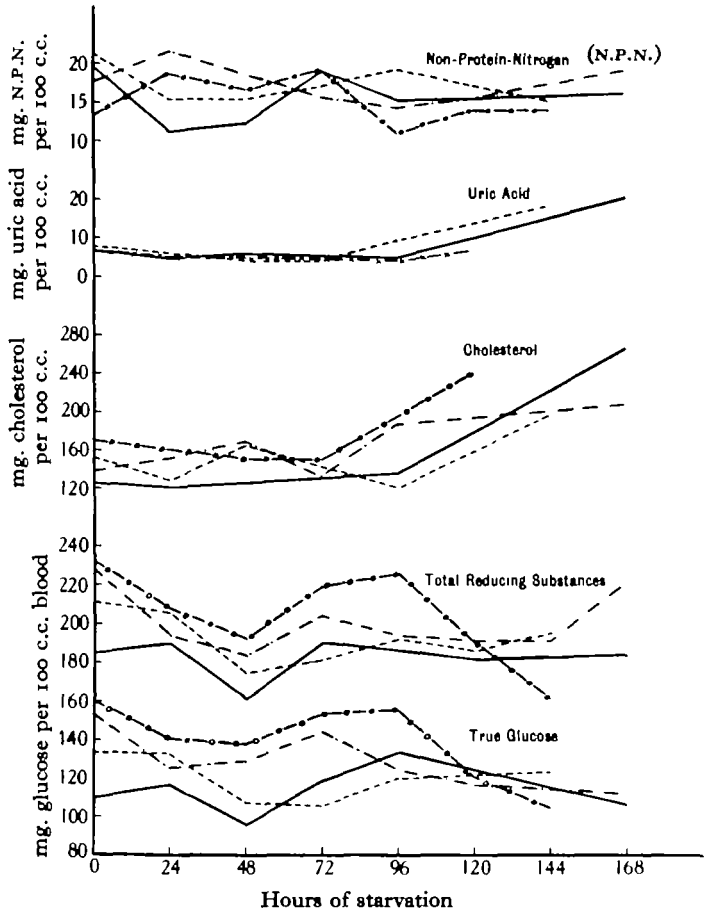


Fig. 1. Variations in certain blood constituents during starvation.

Table I. *Effects of fasting on liver and muscle glycogen and blood lecithin.* (*Averages.*)

Hours of starvation:	0	24	48	72	96	120	144	168
Liver glycogen %	2·68	0·129	0·137	0·193	0·212	0·164	0·159	0·133
Muscle glycogen %	0·278	0·300	0·304	0·228	0·223	0·378	0·099	0·197
No. of cocks	2	4	5	5	5	3	3	2
Mg. lecithin P per 100 c.c. blood	—	18·7	17·48	13·45	17·95	19·25	17·27	16·07
No. of cocks	—	3	4	3	4	2	2	2

at 72 hours and 0·062–0·398 at 96 hours compared with 0·067–0·237 at 48 hours and 0·114–0·258 at 120 hours. From these data alone it would be unjustifiable to draw any inference, but that the liver glycogen was actually increased at 72 and 96 hours is rendered somewhat probable, when it is borne in mind that the blood-

sugar results are in conformity with such a finding. To settle the matter, however, many more experiments would have to be done. Unfortunately, the supply of suitable fowls at the time was inadequate for this purpose. It should also be pointed out that each glycogen value and each lecithin value refers to an individual fowl, whereas the blood sugar and other blood data were obtained on the same fowls throughout the fast. The former results were therefore complicated by individual variations, whereas the latter were not.

If it were assumed that the liver glycogen rises after fasting for 72 or 96 hours, the assumption does not take us very far, because it still leaves unexplained the source of the extra carbohydrate. To throw light on this matter was the purpose of the experiments on the respiratory exchange. But, before describing this work, it is more convenient to give details of other experiments designed to test the carbohydrate metabolism of fowls fasted for varying periods.

SOME EFFECTS OF FASTING ON CARBOHYDRATE METABOLISM.

To test the state of the carbohydrate metabolism, the effects of (*a*) ingestion of glucose, (*b*) injection of insulin, and (*c*) injection of adrenalin, on the blood sugar of fowls fasted for various periods were determined. It was obviously important to concentrate attention on the 72 and 96-hour periods and, for comparison, also on the zero, 24 and 48-hour periods. The same six fowls were used for the glucose and insulin experiments, and five others for the adrenalin tests. The true blood sugar was determined at intervals for 5 hours after administration of glucose and injection of adrenalin and for 11 hours after injection of insulin.

Before carrying out the tests it was essential to know how the blood sugar of fowls, fasted for the above periods, behaved under the experimental conditions. Control experiments were therefore carried out on fowls previously fasted for 0, 24, 48, 72 and 96 hours, the blood sugar being determined at intervals for 11·5 hours. Examples of results for some fowls are given in Table II.

Table II. *Control experiments to determine the influence of the experimental conditions on the true blood sugar of fowls fasted for different periods of time.*

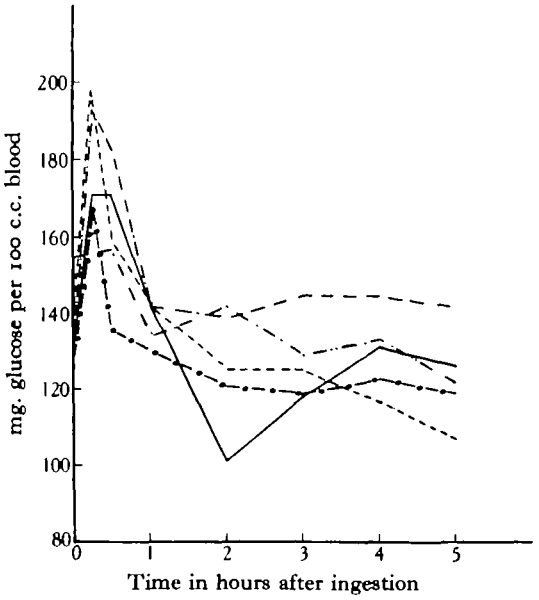
Fowl No.	Length of preliminary fast	Time of sampling after preliminary fast (hours); and true blood sugar (mg./100 c.c.)								
	hr.	0	0·5	1·5	2·5	4·5	6·5	8·5	10·5	11·5
1	0	—	114	104	112	104	98	124	86	119
2	24	105	101	111	105	112	119	114	113	106
3	48	162	138	126	128	121	99	113	103	103
4	72	192	116	113	109	80	124	91	79	78
5	96	158	149	139	130	133	112	117	112	112

The blood sugar tended to remain steady in the 24-hour fasted fowls, and to fall steadily after fasts of 96 hours; while it fluctuated widely in fowls fasted for 0 and 72 hours and less so in those fasted 48 hours. The food in the crop and gizzard of the non-fasted fowls contained, amongst other things, coarse pieces of cellulose.

These, as has been previously shown, were doubtless responsible for the fluctuations in blood sugar after zero hours (Henry, Macdonald and Magee, 1933). The unsteadiness in the values after 72 hours and the gradual decline after 96 hours are in keeping with the fact that the peak in the fasting curve occurs at about these times (Fig. 1), while the variations, in the fowls fasted for 48 hours, seem to be indicative of commencing instability of carbohydrate metabolism.

INGESTION OF GLUCOSE.

A constant amount of glucose, 15 c.c. 0·75*M* solution, was administered into the gizzard by catheter. The blood-sugar curves for each period of fasting, although they had many points in common, were not constant enough to enable one to express the results as an average curve. The findings are, therefore, illustrated by curves from the same fowl at each 24-hour interval of fasting (Fig. 2). These curves are



Starved 0 hours ———; starved 24 hours ------; starved 48 hours —·—·—; starved 72 hours —○—○—; starved 96 hours —··—··—.

Fig. 2. Effect of glucose ingestion on the blood-sugar curve (15 c.c. 0·75 *M*. glucose at 40° inserted into the gizzard).

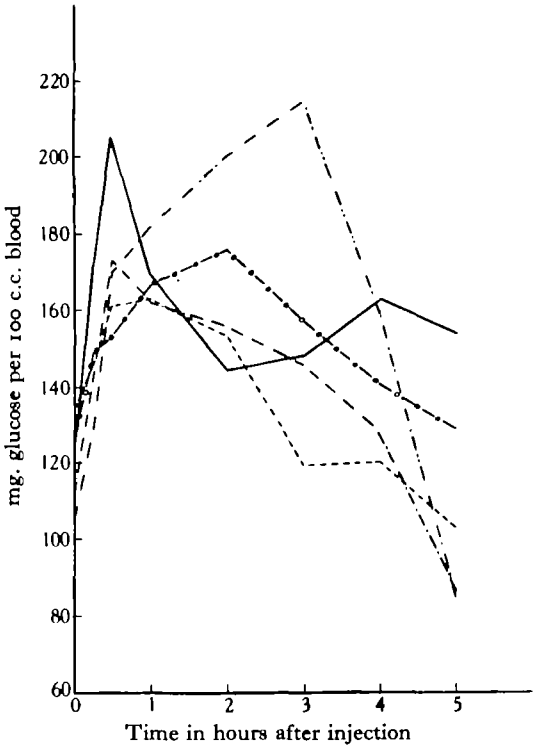
typical of the general trend of the results, but less so at zero and 72 hours than at the other times. The inconstancy found in these curves was expected, because of the irregularity in the corresponding control results (Table II).

Comparison of the curves showed that the hyperglycaemic response was most rapid and marked after fasts of 24 and 48 hours. The response was less marked in the 72-hour curves and still less in the 96-hour curves. In fact, in three out of the six curves at 96 hours the blood sugar scarcely rose at all. This progressive diminution in the degree of hyperglycaemia as the fast proceeded was doubtless causally

related to the fall in the glycogen of the body, and also to the fact that fasting lowers the rate of absorption of glucose (Cori, 1927). It is reasonable to suppose that the less glycogen present in the body, the more rapidly would glucose be removed from the blood to replenish the stores. It would seem that these two factors working in unison would cause the degree of hyperglycaemia to become less as fasting proceeded. It is noteworthy that Macleod (1930) has quoted results showing that, in the mammal, the hyperglycaemic response to a given amount of glucose increases as the length of the previous fast. Judging by the blood-sugar curves the glucose was all absorbed in about an hour in the fasted state. Previous work (Henry, Macdonald and Magee, 1933), indeed, showed that the same amount of glucose was almost entirely absorbed by 48-hour fasted hens in 35 min.

INJECTION OF ADRENALIN.

The fowls were injected intramuscularly with 1 c.c. adrenalin (P.D.) per kg. body weight. Curves were obtained from the same five fowls after each fasting period. Those for one fowl are shown in Fig. 3. The zero-hour curves were irregular; and the inconstancy was very probably traceable to the presence of coarse food in the crop and gizzard, which, as we have seen, causes fluctuations in the blood-sugar



Starved 0 hours ———; starved 24 hours ------; starved 48 hours —·—·—; starved 72 hours —○—○—; starved 96 hours —··—··—.

Fig. 3. Effect of adrenalin injection on the blood-sugar curve (1 c.c. adrenalin intramuscularly per kg. body weight).

curve. The 24 and 72-hour curves have three points in common—the initial rise was more gradual, the maximal increase smaller and the contour flatter than in the 48 and 96-hour curves. The latter resembled each other in general shape except that the 96-hour one showed a much greater relative and absolute increase. The fowls were, therefore, more sensitive to the drug after fasting 96 hours than at any other time. There is no apparent correlation between these results and the liver or muscle-glycogen values. It is, however, important to note that a greater degree of hyperglycaemia occurred in fowls fasted for 96 hours than in non-fasted fowls. The former had an average liver glycogen of 0·212 per cent. (Table I) or of about 0·042 gm. in the entire liver, which weighed about 20 gm. in fowls fasted 96 hours. The volume of blood in the fowl was probably about 150 c.c., and, as the blood sugar in this experiment rose by 110 mg./100 c.c., 165 mg. or 0·165 gm. of sugar were passed into the blood. Only about one-fourth of this sugar could have come from the liver glycogen. The remainder probably came wholly or partially from the muscle glycogen; for, as Cori (1931) has pointed out, adrenalin causes a transference of muscle glycogen to the liver as lactic acid, which the liver re-synthesises to glycogen. The latter process, indeed, can sometimes more than compensate for the liver glycogenolysis, which is the direct cause of the hyperglycaemia. The data do not tell us the total amount of glycogen in the fowl's body after a 96-hour fast and, unless this were known, one could not say whether the body glycogen was entirely responsible for the sugar causing the hyperglycaemia, or whether some of it came from protein or fat. Study of the gaseous exchange after adrenalin would probably be informative. Experiments of this type are being undertaken.

INJECTION OF INSULIN.

The dosage of insulin was 4 units per kg. body weight given intramuscularly. The results were fairly constant so that averages were taken. The mean percentage decreases are shown in Table III.

Table III. *Average percentage fall in blood glucose following injection of 4 units insulin per kg. body weight.*

Hours after injection...	1	2	4	6	8	10	11
Starved 0 hours	36·6	52·0	64·6	67·2	48·1	39·7	34·9
,, 24 ,,	60·7	58·9	55·1	52·3	54·1	35·3	30·0
,, 48 ,,	52·6	66·6	53·8	57·8	46·5	39·4	32·5
,, 72 ,,	51·7	49·9	45·4	45·7	34·3	38·8	24·4
,, 96 ,,	48·7	59·9	60·2	54·6	59·8	44·2	39·9

The zero-hour values were again the most irregular, and it is not, therefore, possible to attach any importance to the apparent insensitiveness of the fowls at an hour after injection. The only other difference between the results of the several tests was, that the 72-hour values showed the fowls to be less sensitive, particularly from the 4th hour after insulin, than at any of the other times. Doubtless, the occurrence, usually at this time, of the peak on the fasting blood-sugar curve was the cause of the insensitivity of the fowls. The most striking thing about these

results, however, is that the degree of hypoglycaemia did not increase as the fast proceeded, which is the case in mammals. Furthermore, convulsions never occurred, the only signs observed being a transitory drowsiness during the first 2 or 3 hours; notwithstanding that the dose of insulin given was more than sufficient to have caused severe convulsions in a rabbit fasted for 24 hours. Another point of interest is the slowness of recovery from the hypoglycaemia, which generally set in between the 6th and 8th hour after insulin and was not completed by the 11th hour. The effect of insulin on the fowl is, therefore, entirely different from what it is in the mammal. This finding confirms the earlier one of Cassidy, Dworkin and Finney (1925), who showed that fowls could endure with impunity doses of insulin of from 4 to 130 units in the fed or fasted state. There is no evidence giving any suggestion as to the cause of this peculiar resistance of fowls to insulin. The problem is one which merits attention.

THE RESPIRATORY EXCHANGE.

The mask employed for collecting the expired air of the fowl has been described by Magee and Reid (1933), and the modified Haldane apparatus, used for the air analysis, by Orr and Magee (1923). The burette of the Haldane apparatus and the dry gas meter employed for measuring the volume of expired air were carefully checked beforehand.

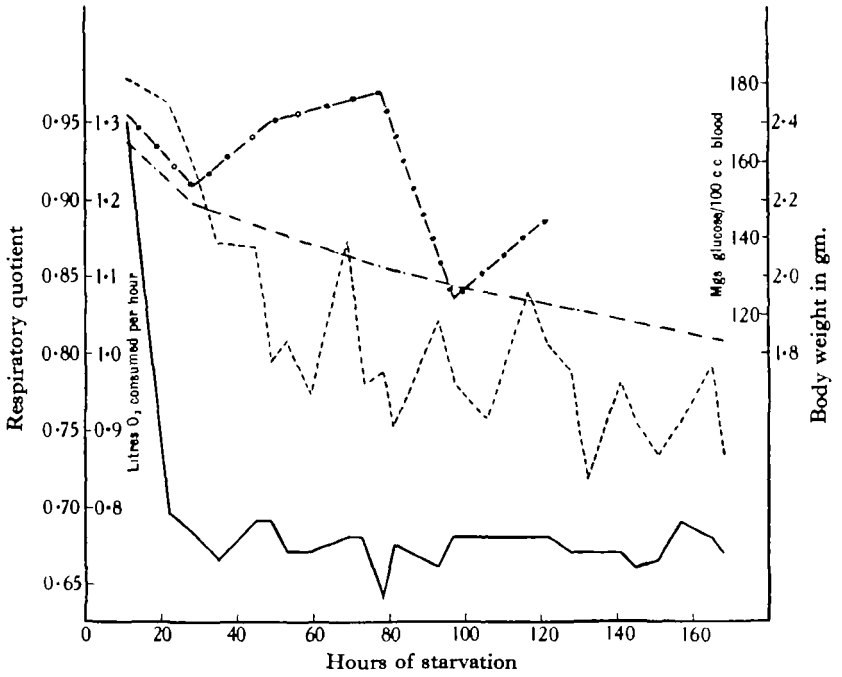
Two adult Rhode Island Red cocks (numbered 1 and 2) were used for these experiments. The comb and wattles were removed in a preliminary operation, and, when the wounds were healed completely, the feathers were removed from the neck, and the fowls trained intensively until constant results were obtained. About 2 weeks generally sufficed for this purpose. Contrary to expectations little difficulty was experienced in maintaining the cocks quiet when the mask was adjusted. Indeed, they have been kept quiet with the mask on continuously for 3 hours, during which samples were withdrawn from time to time. Results have been published showing the reliability of the technique (Magee and Reid, 1933).

EFFECT OF FASTING ON THE GASEOUS EXCHANGE.

The method having been proved to be thoroughly dependable, the fowls were fasted for 7 days, distilled water only being given. Samples of expired air were taken three or four times daily and analysed at once, the first at 6 a.m. and the last at 9 p.m. Blood sugar was also estimated once daily. The procedure was carried out twice on fowl 1 and once on fowl 2; and, as the results agreed very closely, it is only necessary to state the results of one experiment (Fig. 4). For reasons given below it was considered incorrect to calculate the heat production from the Zuntz-Schumburg tables, and the O_2 consumption is stated instead. The O_2 consumption decreased gradually throughout the fast, but it was always highest in the early morning, an observation made also by Benedict and Riddle (1929) on doves and pigeons, and by Benedict, Landauer and Fox on fowls (1932).

The R.Q. was 0·95 11 hours after food was withdrawn. At this time the crop still contained some food, the metabolism of which was doubtless the cause of the

high R.Q. Furthermore, Henry, Macdonald and Magee (1933) have shown that it takes 16–26 hours for the alimentary canal of the fowl to empty itself after a normal meal. The fowls had previously been fed on a mixed diet containing 14 per cent. of protein. This R.Q., which is indicative of carbohydrate metabolism, is higher than what would be expected in mammals fed on a similar diet. The most probable interpretation is that the fowl was transforming carbohydrate into fat. Evidence is given below which substantiates this opinion, and the sudden fall in the R.Q. to 0·695 at 22 hours supports it still further. The R.Q. fell to 0·665 at 35 hours, but



R.Q. ———; O_2 consumption - - - - - - -; blood sugar —○—○—; body weight —·—·—.

Fig. 4.

was 0·69 at 45 and 49 hours; for the remainder of the fast it was no higher than 0·68 except at 157 hours, when it was 0·69. The blood sugar attained its maximum of 178 mg. at 78 hours, and the R.Q. was then at its lowest, 0·64, while at 49 and 81 hours it was 0·69 and 0·675 respectively, the corresponding blood sugars being 170 and 167 mg. In the second cock the R.Q. was slightly higher throughout. It was 0·92 at 4 hours and fell gradually to 0·695 at 48 hours, and thereafter varied from 0·68 to 0·71 with an average of 0·695 until the end of the experiment. The blood sugar in this fowl did not show a definite peak, but a plateau, which, as the figures given below indicate, lasted from the 48th until the 102nd hour:

Hours of fasting...	29	48	71	76	94	102	120
Blood sugar	167	183	183	190	188	190	154
R.Q.	0·725	0·695	0·695	0·680	0·705	0·680	0·695

The reason for this divergence from the other results is far from clear, and it can be seen that the data do not confirm those in Fig. 4, in that the high blood-sugar values were not always accompanied by lower R.Q.s than the preceding and following ones. Had this been so the suggestion raised by the data from cock 1 (Fig. 4) that the rise in blood sugar was due to conversion of fat into carbohydrate would have been supported. The variation in the results, however, makes such a conclusion impossible. The failure of the method to give a direct answer to the question is not to be wondered at, because of the numerous factors involved; and for this reason the data just quoted, while they do not afford any evidence showing that fat can be converted into sugar, cannot be regarded as denying the possibility of such a change taking place.

The most remarkable thing about all these results is that the R.Q. remained below that of fat oxidation from the 48th hour, or earlier, until the end of the fast. R.Q.s of 0·68 have also been recorded by Benedict and Riddle (1929) in pigeons fasted for 30 hours using a chamber method, and Benedict *et al.* (1932) obtained values of 0·66–0·68 in fowls fasted 40 hours with the same technique. Clearly the cause was not fat oxidation, either direct, or indirect by prior conversion into sugar. Nor was the low R.Q. due to incomplete fat oxidation. The urine was tested for ketone bodies repeatedly throughout the fast, and always the results were negative. It is also noteworthy that Needham (1933) was unable to detect ketone bodies in the egg of the developing chick embryo. The fact that a large proportion of the nitrogenous end-products in the fowl are excreted as uric acid, suggested that the low R.Q. might be associated with the metabolism of protein. Uric acid is richer in O_2 than urea, and the combustion R.Q. of amino acids to uric acid should therefore be lower than when urea is the final nitrogenous product. The subjoined equations demonstrate that this is so:

$$2CH_3.CHNH_2.COOH + 6O_2 = (NH_2)_2CO + 5CO_2 + 5H_2O$$

Alanine — Urea

$$\text{R.Q.} = 5/6 = 0{\cdot}833$$

$$8CH_3.CHNH_2.COOH + 21O_2 = 2C_5H_4N_4O_3 + 14CO_2 + 24H_2O$$

Alanine — Uric acid

$$\text{R.Q.} = 14/21 = 0{\cdot}667$$

The fact that the R.Q. remained practically constant throughout the 7 days' fast suggested that the uric acid quota of the total nitrogen excretion would also remain fairly steady during a fast of the same duration. An artificial anus was therefore established, so that the urine was excreted by the cloaca and the faeces by the new opening. Two fowls were operated on successfully, but normal defaecation never occurred, as the intestinal musculature at the artificial opening was always in a state of spastic contraction, which was not lessened by daily intestinal lavage. Although the fowls remained fairly healthy, they lost weight so rapidly that they had to be killed. There was therefore no alternative but to collect the urine, discontinuously, for short periods during the fast. Cock 2 was fasted for 7 days and the uric acid/total nitrogen ratio of the urine determined two or three times daily. In obtaining the

sample of urine the fowl was given 50 c.c. water by catheter, then strapped on a special board inclined at an angle of 45°, and the cloaca, oral to the entry of the ureters, blocked with a cotton-wool plug. The diuretic effect of the water soon became evident and a glass tube about 1 cm. in diameter was then inserted into the cloaca, and the urine collected for 35 min., during which 20–40 c.c. were generally excreted. Some of the values obtained are given in Table IV.

Table IV. *Urinary uric acid as percentage of total nitrogen in fowl fasted for 7 days.*

Hours of fasting...	17	41	68	114	137	161	169
$\frac{\text{Uric acid } N_2}{\text{Total } N_2} \times 100$	55	54	56	58	57	52	45

Except for a slight fall at the end of the fast the proportion of uric acid remained very constant. This result is in agreement with the above interpretation of the low R.Q. during the 7 days' fast.

EFFECT OF PROTEIN INGESTION.

The foregoing result suggested that protein ingestion would not appreciably alter the fasting R.Q. This was shown to be the case in several experiments in which egg-white was administered by catheter into the gizzard. An example of the results obtained is given in Table V.

Table V. *Effect of administration of 15 gm. egg-white into the gizzard on the gaseous exchange of fowl 1, previously fasted 72 hours.*

Min. after ingestion...	0	30	60	90	120	210	300
R.Q.	0·685	0·655	0·720	0·720	0·660	0·650	0·660
O_2 consumption (litres per hour)	1·020	1·149	1·120	—	1·170	1·243	1·111

The R.Q. usually rose slightly during the first hour after ingestion, but in some experiments it remained practically constant, while the fact that the O_2 consumption remained up for 4 hours or more, indicated that the protein was being metabolised. The highest R.Q. obtained in any of these experiments was 0·72, whereas in mammals the metabolism of the same food would doubtless have resulted in one of about 0·80. In order to ascertain the effect on the R.Q. of prolonged ingestion of protein, fowl 2 was fed for several days on coagulated egg-white, casein and fish meal, and the R.Q. determined in the fasting and non-fasting state. Values greater than 0·697 were never obtained. There is therefore no doubt that the metabolism of protein in the fowl is associated with an R.Q. of about 0·69 or less.

There still remained a doubt as to whether the uric-acid quota in the urine of fasting fowls is altered by ingestion of protein. Experiments were accordingly carried out to determine the influence of protein ingestion on the uric acid/total nitrogen ratio in the urine. The following data illustrate the nature of the results obtained:

Fowl fasted 48 hours and given 15 gm. egg-white by catheter.

Hours after feeding...	0	1	2	4	6
Uric acid / Total N_2 × 100	43	41	32	28	28

Each sample of urine was collected for 40 min. The ratio fell slightly but, as the urea was not determined owing to the small amounts of urine available, it was not possible to say whether the fall in the ratio was due to an increase in the urea quota. If this were so it would, presumably, account for the slight rise which sometimes occurred in the R.Q. after protein ingestion (Table V). The matter was not, however, considered sufficiently important to pursue any further at the time. The uric acid-total N_2 ratio found in these experiments falls below the range of values obtained by Coulson and Hughes (1930), 54–77 per cent., but within that of the observers quoted by them, 30–70 per cent.

There can be no doubt, in view of the above findings, that the low R.Q. of fowls in the fasting state, or when metabolising protein, is due to the large amounts of uric acid which they normally excrete in the urine. Uric acid contains, as we have seen, a higher proportion of O_2 than urea, so that the fowl, as compared with the mammal, excretes a larger proportion of its O_2 intake in non-gaseous form. It is therefore fallacious to employ the Zuntz-Schumburg tables for calculating the heat production in fowls; because, as has been shown, the R.Q. of protein metabolism in the mammal, 0·8, corresponds with one of about 0·69 in the fowl.

EFFECT OF FAT INGESTION.

These experiments provided further evidence in support of our conception as to the cause of the low fasting R.Q. As this was always found to be below 0·707 it was anticipated that it would be raised slightly during metabolism of a pure fat meal. The results (Table VI) confirmed our expectations.

Table VI. *Effects of meals of pure fat on the respiratory exchange.*

Min. after feeding...	0	30	60	90	120	210	270	
R.Q.	0·665	0·730	0·720	0·680	0·690	0·705	0·715	Fowl fasted 60 hours. 5 c.c. olive oil given by catheter
O_2 consumption, litres per hour	1·303	1·378	1·392	—	1·347	1·449	1·474	
R.Q.	0·670	0·700	0·710	0·760	0·760	0·740	0·725	Fowl fasted 24 hours. 10 c.c. melted butter given by catheter
O_2 consumption, litres per hour	1·228	1·248	1·251	1·382	1·248	1·220	1·328	

The R.Q. was raised during the metabolism of both fats, the increase being greater with butter than with olive oil. The difference is doubtless attributable to the fact that butyrin has a higher combustion R.Q. than olein:

$$2C_3H_5(C_4H_7O_2)_3 + 37O_2 = 30CO_2 + 26H_2O$$

Butyrin

R.Q. $= 30/37 = 0{\cdot}81$

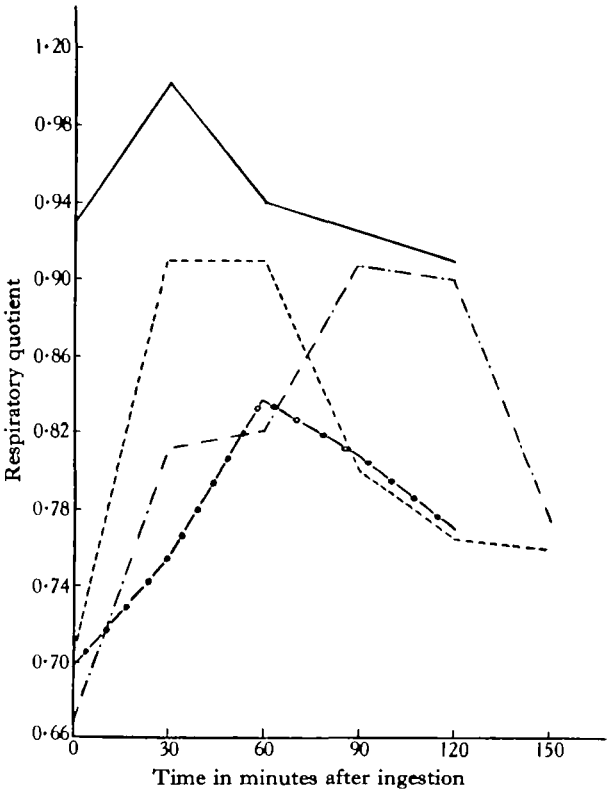
$$C_3H_5(C_{18}H_{33}O_3) + 80O_2 = 57CO_2 + 52H_2O$$

Olein

R.Q. $= 57/80 = 0{\cdot}712$

EFFECT OF CARBOHYDRATE INGESTION.

There were two objectives in this research: (*a*) to obtain further evidence than that given in Fig. 4 that the fowl readily converts carbohydrate into fat, and (*b*) to compare the effects of glucose ingestion, after varying periods of fasting, on the R.Q. with those on the blood-sugar level.



Starved 0 hours ————; starved 24 hours ---------;
starved 48 hours —·—·—; starved 72 hours —○—○—.

Fig. 5. Respiratory quotient after glucose ingestion (25 c.c. 25 % at 40° C. placed in gizzard).

Varying amounts of glucose were fed by catheter into the gizzard of both fowls in the unfasted state and the R.Q. determined at intervals afterwards. The maximal values were reached at different times after giving the glucose. In five experiments on the two fowls the maxima were: 0·945, 0·947, 0·951, 1·016 and 1·019; in five experiments carried out on the fowls in the fed state (stock diet), without giving

glucose, the R.Q.s obtained were 0·878, 0·888, 0·954, 0·995 and 1·036. It is quite clear from these results that the fowl during luxus consumption of carbohydrate can readily convert it into fat.

For the second objective the fowls were previously fasted for 0, 24, 48 and 72 hours, and then 25 c.c. 25 per cent. glucose were inserted into the gizzard by catheter. A test at 96 hours could not be carried out, because the fowls invariably had diarrhoea

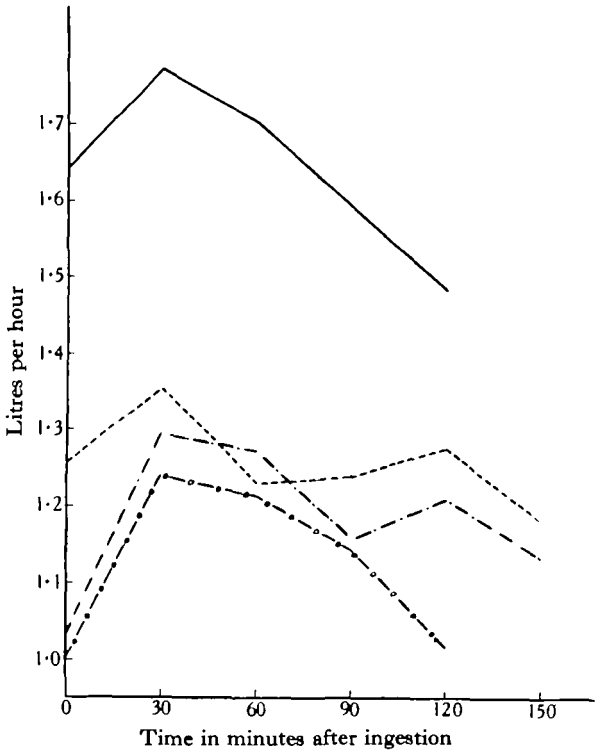


Fig. 6. Oxygen consumption after glucose ingestion (25 c.c. 25 % at 40° C. placed in gizzard).

after receiving the glucose. Several experiments were performed and, as the results were all of the same general type, those shown in Fig. 5 are given as an example.

The R.Q. after glucose administration to fowls in the unfasted state attained a much higher maximum and fell more rapidly than when previously fasted, and, as the highest value was 1·02, this may be taken as additional evidence of fat deposition. The maxima of the 24 and 48-hour curves were about equal, but the increase was much slower in the latter. The 72-hour curve showed an equally slow rise to a peak of only 0·84. The corresponding O_2 consumption values are shown in Fig. 6. When considered along with the R.Q.s they indicate that the oxidation of the absorbed glucose decreased with increase in the duration of the previous fast. The immediate cause of this effect was most probably the same as that to which was attributed the progressive decrease in hyperglycaemia after glucose ingestion brought

about by increase in the length of the previous fast (Fig. 2). That is, that as the period of fasting increased the rate of absorption fell off, and more and more of the glucose which was absorbed was utilised to re-establish the depleted glycogen stores.

SUMMARY.

The occurrence of a peak in the true blood-sugar curve of the fasting fowl on the 3rd or 4th day, as found in previous work, was confirmed. No correlation was found to exist between this increase in blood sugar and the concentration of protein (uric acid and non-protein nitrogen) or fat (cholesterol and lecithin) metabolites in the blood, or between it and the muscle glycogen; in regard to liver glycogen and the R.Q. the results were inconclusive. After glucose ingestion the hyperglycaemic response and the rate of oxidation of sugar decreased as length of the previous fast was increased. These effects were probably due to a falling off in the rate of absorption and to utilisation of increasing proportions of absorbed sugar to restore the glycogen deposits. Adrenalin produced a greater degree of hyperglycaemia in fowls fasted for 96 hours than for shorter periods, and the corresponding liver glycogen values indicated that only about one-fourth of the extra sugar could have come from the original glycogen stores.

The R.Q. during luxus absorption of carbohydrate was near or above unity, thus indicating conversion of sugar into fat. The R.Q. rapidly fell to below 0·700 and then remained steady during a fast of 7 days; the fasting quotient was not appreciably altered by protein meals or by maintenance on a protein diet, but was raised by ingestion of pure fat. Uric acid formed, during fasting, 50 per cent. or more, and after a protein meal 30 per cent. or more of the urinary nitrogen, and the low R.Q. is believed to be due to the loss of O_2 incurred in the synthesis of uric acid. The Zuntz-Schumburg tables are, therefore, inapplicable to the fowl. In fasting, ketosis did not occur, and the metabolic rate was higher in the morning than in the evening.

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