THE ENERGY-SOURCES IN ONTOGENESIS

III. THE AMMONIA CONTENT OF THE DEVELOPING AVIAN EGG AND THE THEORY OF RECAPITULATION

By JOSEPH NEEDHAM, M.A., Ph.D.,
Fellow of Gonville and Caius College, Cambridge.

(From the Biochemical Laboratory, University of Cambridge.)

(*Received 15th May* 1926.)

(With Five Text-figures.)

INTRODUCTION.

In the preceding papers of this series (16, 17) it was shown that during the course of development in the hen's egg, the intensity of production of urea and uric acid reached a maximum about the second quarter of incubation. There was, therefore, a point of maximum protein combustion in the ontogenetic process. It was shown that this maximum point occurred between the time specially associated with carbohydrate combustion and the time specially associated with the combustion of fat.

Besides urea and uric acid, however, there is one other form in which nitrogen could be got rid of, *i.e.* ammonia. It has long been known, especially since the work of Krogh (10), that no nitrogen is lost as gaseous ammonia during development, but the ammonia produced might rapidly combine with any acid present near the site of deamination. It was therefore necessary to check the figures in the preceding papers by determinations of the ammonia excretion of the embryo. For it was conceivable that in the very early stages the ammonia excretion might be large enough to affect materially the shape of the curve for the total protein catabolised each day.

No previous determinations of ammonia in the hen's egg exist in the literature, except those of Aggazzotti (1) which are not helpful for our purpose because they were done after total hydrolysis of the proteins in the various fractions.

TECHNIQUE.

The eggs used in this work were all laid by White Leghorn hens and incubated under standard conditions, *i.e.* temperature constant at 38·8 ± 0·4° C., humidity constant at 67·5 ± 2·5 per cent., and continuous ventilation by warm air. The eggs were aired every morning for 15–20 minutes and rolled once a day. The extractions were done exactly as described in the first paper of this series (16).

The estimations of ammonia were performed by the same method as was used for the urea, that of Folin and Wu (6), omitting the urease. A saturated solution of

borax is added to a portion of the extract of the embryos and their membranes, and the ammonia rapidly distilled over into ice-cold 0·05 *N* hydrochloric acid, which is estimated colorimetrically by nesslerisation.

The figures for calculating the percentages on the basis of wet and dry weights of embryo were taken from the work of Murray(15), who made his measurements in part at any rate on chicks of the same breed from the same farm as those used in this research.

EXPERIMENTAL RESULTS.

The experimental results are given in Table I. It will be seen that the estimations do not cover the whole of the developmental period, but that they do cover all that time which we know from the urea and uric acid curves to be most important in the combustion of protein by the embryo. Column 1 gives the time of development, column 2 the number of embryos used for the determination, and column 3 the amount of ammonia in mg. per embryo. A total of 238 eggs were used for

Table I.

1	2	3	4	5	6
Day	No. of embryos used	mg. per embryo	Free ammonia		mg. per embryo: smoothed curve for calculation of amounts excreted per day
			mg. % wet weight	mg. % dry weight	
0	—	—	—	—	—
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	—	—
4	55	·00326	2·963	55·25	·00150
5	28	·00364	1·647	30·98	·00364
6	22	·0046	1·088	19·50	·00580
	15	·0071	1·680	30·10	
7	17	·0076	1·034	17·67	·00940
	12	·0131	1·782	30·47	
8	17	·0095	0·798	12·86	·01400
9	13	·0250	1·336	21·16	·01950
	13	·0235	1·293	19·9	
10	13	·0247	0·928	13·26	·02500
11	11	·0282	0·752	9·75	·03250
12	8	·0389	0·762	8·65	·03950
13	8	·0556	0·813	8·05	·04850
14	6	·0508	0·566	4·62	·06000
15	—	—	—	—	—
Total of eggs used	238				

establishing this curve. In the next column, column 4, are shown the figures for ammonia content in mg. per cent. of wet weight of embryo, and in column 5 the same, but calculated for dry weight of embryo. Lastly, in column 6 are placed the values found by smoothing the curve of column 3 so that the average amount excreted by the embryo each day can be calculated.

Column 3 is shown graphically in Fig. 1. The points lie on a well-defined curve,

which is practically the same as regards its slope as those previously found for urea and uric acid ((16), p. 202 and (17), p. 118), but it will be seen that the total amounts with which we are now dealing are very much smaller than those of the urea curve and infinitesimal compared with the amounts of uric acid which the embryo produces. Columns 4 and 5 of Table I are shown graphically in Fig. 2. Inspection of this graph shows that the ammonia present in 100 gm. of embryo, whether considered as wet or dried, falls steadily from the beginning of development. The process is, as might be expected, more pronounced in the dry weight curve because as the embryo grows it becomes less wet. Whether the point at the fourth day

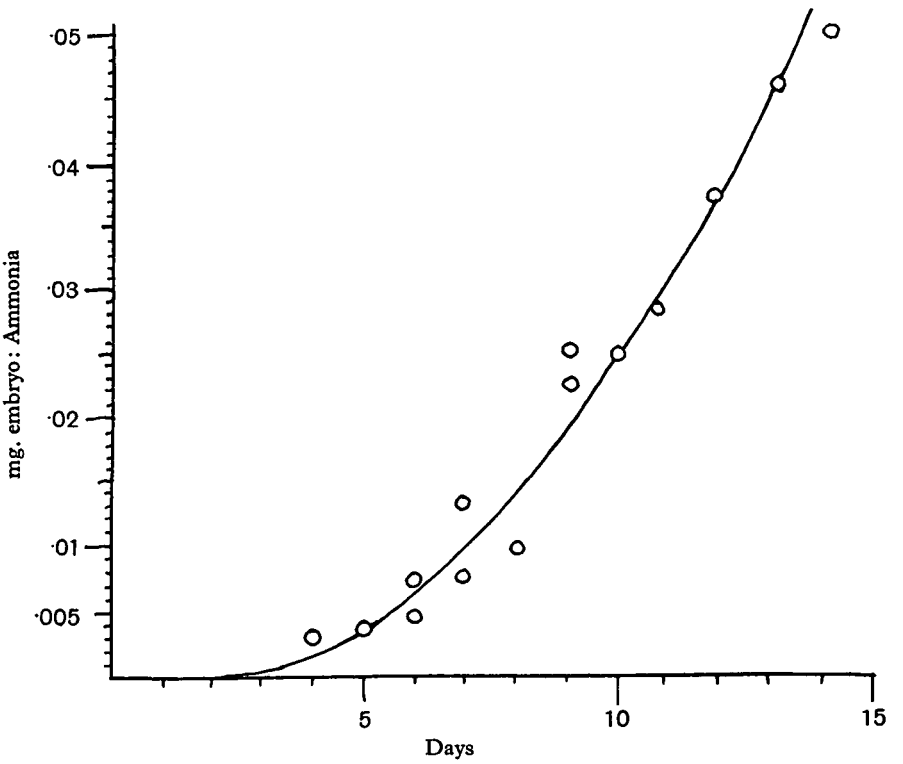

Fig. 1.

represents a peak or a descent from a yet higher value, is as yet uncertain. In Fig. 3 the amounts of ammonia, urea and uric acid, expressed as mg. per cent. of dry weight of embryo have all been placed on the same graph. Here the comparison becomes very interesting, for just as urea was previously found to rise to its maximum two days before uric acid, so now we find ammonia at its maximum (or, more correctly, higher than at any other time so far determined) five days before urea. This important point will be referred to again in the discussion.

The question which has not yet been answered is whether the ammonia excreted by the embryo will make any difference to the curve for total protein combusted each day, as given in the preceding paper. Table II gives the figures relevant to

Table II.

1	2	3	4	5	6	7	8	9
Day	mg. ammonia excreted per day per embryo		mg. NH_3-nitrogen excreted per day per embryo	mg. urea and uric acid N excreted per day per embryo	mg. total N excreted per day per embryo	mg. protein combusted		
	Experim.	Smoothed				per day per embryo	% wet weight	% dry weight
0– 1	—	—	—	—	—	—	—	—
1– 2	—	—	—	—	—	—	—	—
2– 3	—	—	—	—	—	—	—	—
3– 4	—	—	—	—	—	—	—	—
4– 5	·00214	·00180	·00148	·00234	·00382	·0239	14·48	270·8
5– 6	·00216	·00250	·00206	·00265	·00471	·0294	9·13	166·4
6– 7	·00360	·00330	·00273	·0099	·01263	·0789	13·63	236·7
7– 8	·00460	·00410	·00338	·0802	·08358	·5225	54·31	895·0
8– 9	·00550	·00500	·00412	·1840	·1881	1·1750	78·20	1226·0
9–10	·00550	·00590	·00486	·2553	·2611	1·6320	72·90	1072·0
10–11	·00750	·00700	·00576	·3428	·3486	2·178	67·96	917·3
11–12	·00700	·00830	·00683	·4389	·4457	2·786	62·91	754·7
12–13	·00900	·00980	·00807	·5538	·5619	3·512	58·80	616·0
13–14	·01150	·01150	·00947	·6847	·6942	4·339	54·89	485·1
14–15	—	—	—	·8264	—	—	—	—
		Total	·04876					

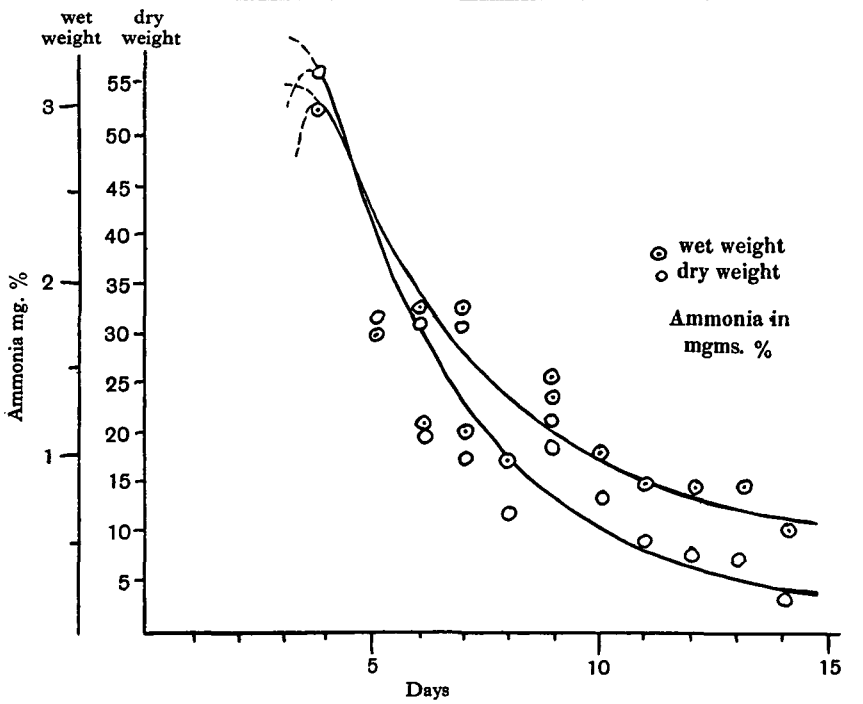

Fig. 2.

this matter. Column 1 shows the time of incubation, columns 2 and 3 the mg. of ammonia excreted per day per embryo, column 4 the mg. of ammonia nitrogen excreted per day per embryo, and column 5 the mg. urea N and uric acid N excreted per day per embryo. From these it is simple to calculate the mg. total N excreted per day per embryo, as shown in column 6, and thence the mg. protein combusted per day per embryo, per day per cent. wet weight, and per day per cent. dry weight. These will be found in columns 7, 8 and 9.

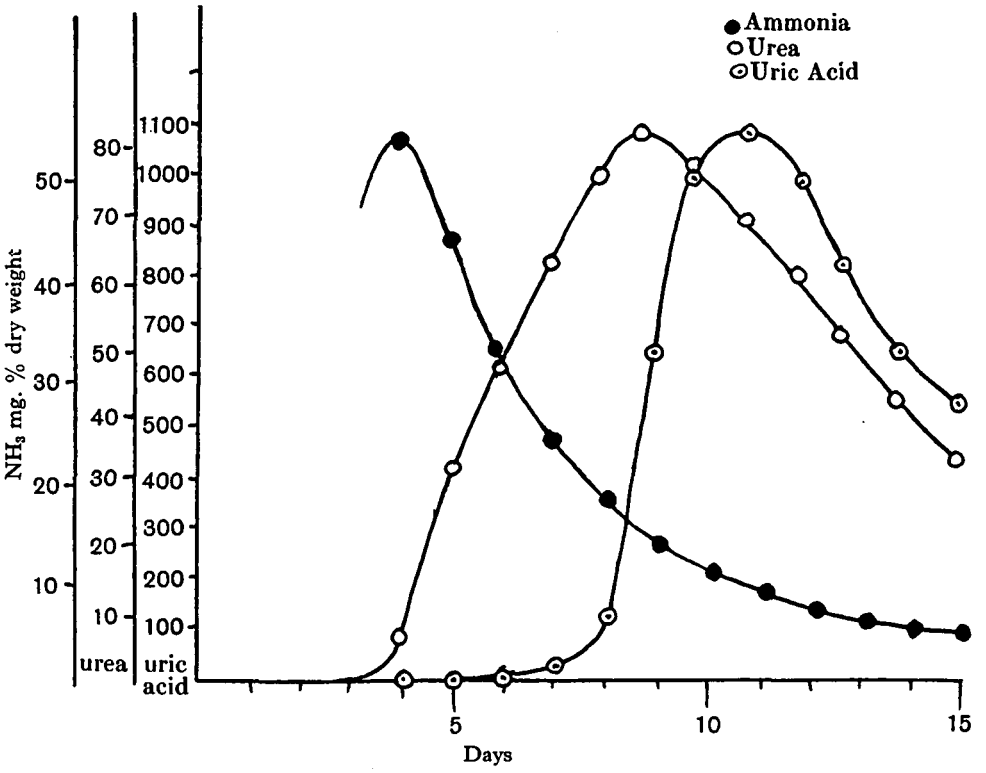

Fig. 3.

Fig. 4 shows the data of column 9 graphically. The correction for ammonia production is clearly seen to be negligible except in the first quarter of incubation, and its effect then is to double the amount of protein combusted. This, however, in no way alters the general shape of the curve, and the peak on the eighth day remains unaffected. It is interesting to note that 100 gm. dry weight of embryo combust on the fourth day of development as much as they do on the twentieth, yet between these two dates there is a point at which they combust six times as much in the same period of time. The combustion of protein therefore goes on during both the carbohydrate and fat periods, but not to anything like the same extent as it does when the former is passing over into the latter.

In the light of the figures for the ammonia it will be of interest to examine again the partition of excreted nitrogen among the nitrogenous end-products.

Table III.

Excretion of nitrogen.

Day	mg. excreted per embryo per day expressed as percentages of total N excreted per embryo per day		
	Ammonia	Urea	Uric acid
0– 1	—	—	—
1– 2	—	—	—
2– 3	—	—	—
3– 4	—	—	—
4– 5	38·8	55·49	5·71
5– 6	43·74	48·83	7·43
6– 7	21·45	54·75	23·8
7– 8	4·04	15·80	80·16
8– 9	2·18	9·19	88·63
9–10	2·02	8·58	89·40
10–11	1·68	8·43	89·89
11–12	1·14	7·96	90·90
12–13	1·45	7·79	90·76
13–14	1·36	7·39	91·25
14–15	—	—	—

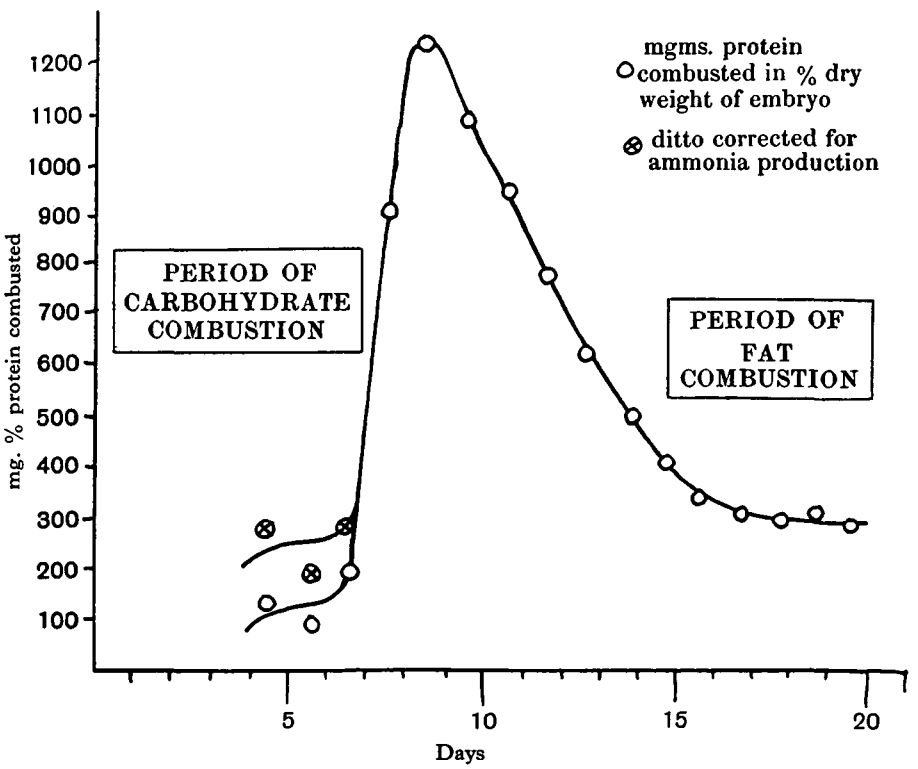

Fig. 4.

Table III gives the figures which enable us to do this, and Fig. 5 shows the relations between them. The uric acid rises steadily to its adult value in very much the same way as before, but the urea never attains 90 per cent. of the total excretion, for it shares almost its highest figures with ammonia from the fourth to the seventh day. In the later period the uric acid seems to gain rather at the expense of the ammonia than of the urea.

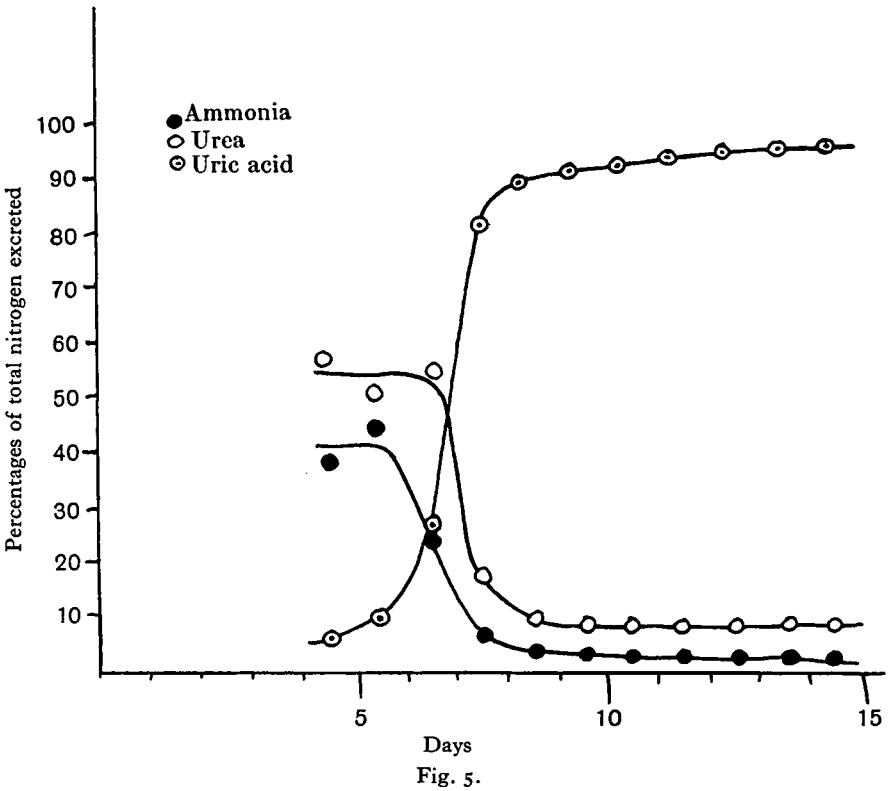

Fig. 5.

DISCUSSION.

The chief point of importance which seems to be brought out by the experiments recorded in this paper is the time-relation between ammonia, urea and uric acid during ontogenesis. Table IV gives some idea of the process. The nitrogenous excretory product which has the smallest molecular weight and the highest nitrogen percentage reaches its maximum earliest in ontogenesis, that which has the largest molecular weight and the smallest nitrogen percentage reaches its maximum latest. The simplest product of deamination is the first to appear, the most complicated is the last. Yet the latter accounts for 91·5 per cent. of the total nitrogen excreted by an embryo throughout its development, and the former for only 1 per cent., while the intermediate compound, urea, accounts for 7·5 per cent.

General biological considerations are at all times dangerous if they are not carefully kept close to known facts, but in this case it is difficult to resist the opinion

that in the ammonia-urea-uric acid succession we have to deal with a recapitulatory phenomenon. The theory of recapitulation, associated with the great name of von Baer and Darwin, has been of great service as a stimulant to morphological research, though untenable in its extremer forms. But in biochemistry, its applications have so far been few. The cause of this lies probably in the fact that general information as regards comparative biochemistry has always been meagre. The morphologist had an infinitude of forms, embryonic and adult, with which to compare the embryonic stages of any given animal, but biochemists have had, as it were, no such base-lines. There are, however, certain correlations in which the biogenetic law has been found to hold in chemical embryology:

1. Bunge (4) noticed that the vertebrate embryo is richer relatively in sodium chloride than the newly-born animal and that after birth it becomes even poorer in sodium and chlorine. Liebermann (11) made similar observations as regards the ash of the chick embryo, and these have recently been confirmed by Murray (15). Bunge and Macallum (12) have interpreted these results as indicating the shadow of a remote marine ancestor. In the chick the ash content is lowered by half in the first fifteen days of development.

Table IV.

	Molecular weight of compound	Percentage of nitrogen in the compound	Time of peak during development of chick (in days)	Absolute mg. nitrogen excreted during the whole development of the chick	Percentage of the total nitrogen excreted during the whole development of the chick
Ammonia	17	82·3	4	0·120	1·07
Urea	60	46·6	9	0·843	7·58
Uric acid	168	33·3	11	10·161	91·35
			Total	11·124	

2. It is possible that a similar argument might be applied to the fact that during embryonic growth the water-content uniformly decreases. This is an absolutely general law.

3. Wells and Corper (24), Mendel and Mitchell (13), and Jones and Austrian (9) found that during embryonic growth the adult battery of purine enzymes was only gradually formed. Pig embryos less than 150 mm. long possess no nucleases at all; from 150–170 mm. guanase first appears followed after a time by adenase, and lastly, when the embryo is longer than 170 mm. xanthineoxidase makes its appearance. The sequence of purine enzymes thus repeats what has been found to hold in phylogeny. Straughan and Jones (22) found only guanase in the yeast-cell, and no adenase, xanthineoxidase, or uricase, while in the mollusc, *Sycotypus canaliculatus*, Mendel and Wells (4) found guanase and adenase, but no xanthineoxidase and no uricase.

4. Steudel and Osato (21) found that the membrane of eggs such as that of the herring cannot be keratin and approaches in composition to a mucin. They suggested that the ovomucoid which occurs in the white of the hen's egg might

be considered a phylogenetic reminiscence of the time when it was used for the purpose of a membrane, as it is now in fishes and amphibia.

5. The lower animals have, broadly speaking, no power of maintaining a constancy in their internal environment. Similarly the early stages of embryonic life show a lack of the power of independence. As regards osmotic pressure this has been found to be the case by Backmann and Runnström (2) and Przylecki (19) for the frog, and by Bialascewicz (3) for the chick. The evidence for this is not as satisfactory as it might be, for Backmann and Runnström considered the egg as a whole rather than the embryo in their estimations. On the other hand, there is no doubt that the power of regulation of heat-production only develops at a late stage in embryonic life. Pembrey, Gordon and Warren (18) found that the embryonic chick behaved like a cold-blooded animal till nearly the end of incubation. Giaja (7) has recently confirmed this for the rabbit, showing that the thermogenetic margin increases constantly with age.

6. The succession in the kinds of nitrogenous excretory products reported in the present paper may have a recapitulatory significance. Bacteria excrete ammonia, as may be seen from numerous references and in the monograph of Hirsch (8), and so do yeasts, according to Ehrlich (5). The same method of excreting nitrogen is used by insect larvae if we accept the work of Weinland (23) on *Calliphora vomitoria* and Sosnoffski (20) on *Lucilia caesar*. The birds would seem to have gone off along a reptilian side-line in the selection of uric acid as their principal nitrogenous end-product.

7. It is possible that a recapitulatory significance may also attach to the general succession of energy-sources during ontogenesis which has been described in this series of papers. The order carbohydrate, protein, fat, besides being the sequence in which these foodstuffs are selected by the developing embryo for combustion, is also that in which they can be arranged according to solubility in water, hydrolysis by digestive enzymes in the intestinal tract, and simplicity of synthesis by solar energy. A fuller discussion of the speculations which arise out of these facts will be found in the paper of Murray (15).

In future work, it will be important to determine whether ammonia production precedes urea and uric acid production in all embryos or whether this phenomenon is limited in its extent.

SUMMARY.

1. Investigation of the ammonia content of the developing hen's egg shows that though in absolute amount it steadily increases during incubation, in percentage of the embryonic weight it declines.

2. The intensity of production of ammonia reaches its highest point on the fourth day, *i.e.* five days before that of urea production, and seven days before that of the production of uric acid.

The absolute amounts of nitrogen excreted in the form of ammonia are so small, however, that the curve for protein combusted by 100 gm. of embryo each day is hardly affected, and rises to a peak between the eighth and ninth days.

4. These results are compared with others already in the literature of chemical embryology, which seem to bear on the theory of recapitulation. They add further support to the conception of an ontogenetic succession of energy-sources.

The thanks of the writer are due to Prof. Sir Frederick Hopkins for his constant interest, to Miss M. Stephenson for certain valuable suggestions, and to the Government Grant Committee of the Royal Society for a grant which partially defrayed the cost of these researches.

REFERENCES.

(1) AGGAZZOTTI, A. (1919). *Archivio d. Scien. Biol.* **1**, 120.
(2) BACKMANN, A. and RUNNSTRÖM, J. (1912). *Pflüger's Archiv*, **144**, 287.
(3) BIALASCEWICZ, K. (1912). *Archiv f. Entwicklungsm.* **34**, 489.
(4) BUNGE, G. (1889). *Lehrbuch d. phys. u. path. Chem. Leipzig.*
(5) EHRLICH, P. (1907). *Ber. d. d. chem. ges.* **40**, 1027.
(6) FOLIN, O. and WU, W. (1919). *Journ. Biol. Chem.* **38**, 94.
(7) GIAJA, J. (1925). *Ann. phys. et phys.-chem. Biol.* **1**, 628.
(8) HIRSCH, H. (1918). *Die Einwirkung v. Mikroorganismen a. d. Eiweisskörper.* Berlin, Borntrager.
(9) JONES, E. C. and AUSTRIAN, C. (1907). *Journ. Biol. Chem.*.**3**, 227.
(10) KROGH, A. (1906). *Skand. Arch. f. Physiol.* **18**, 364.
(11) LIEBERMANN, L. (1888). *Pflüger's Archiv*, **43**, 105.
(12) MCCALLUM, A. B. (1926). *Physiol. Rev.* **6**, 319.
(13) MENDEL, E. S. and MITCHELL, L. (1907). *Amer. Journ. of Physiol.* **20**, 115.
(14) MENDEL, E. S. and WELLS, G. (1909). *Amer. Journ. of Physiol.* **24**, 170.
(15) MURRAY, H. A. (1926). *Journ. Gen. Physiol.*
(16) NEEDHAM, J. (1926). *Brit. Journ. Exp. Biol.* **3**, 189.
(17) —— (1926). *Brit. Journ. Exp. Biol.* **4**, 114.
(18) PEMBREY, M. S., GORDON, G. and WARREN, H. (1895). *Journ. Physiol.* **17**, 331.
(19) PRZYLECKI, ST J. (1921). *Trav. Physiol. Lab. Instit. Nencki*, Warsaw, **1**, No. 4.
(20) SOSNOFFSKI, S. (1902). *Bull. Ac. Sci. Cracovie*, p. 568.
(21) STEUDEL, O. and OSATO, G. (1923). *Zeitschr. f. Physiol. Chem.* **127**, 220.
(22) STRAUGHAN, B. and JONES, E. C. (1909). *Journ. Biol. Chem.* **6**, 245.
(23) WEINLAND, E. (1906). *Zeitschr. f. Biol.* **47**, 232.
(24) WELLS, G. and CORPER, M. (1909). *Journ. Biol. Chem.* **6**, 469.