Estimation of protein reserves and the nitrogen content of organs in protein-depleted and repleted cocks*

By JACOBUS P. H. WESSELS and HANS FISHER

Nutrition Laboratories, Department of Animal Sciences, Rutgers, The State University, New Brunswick, New Jersey, USA

(*Received* 16 *June* 1964—*Accepted* 10 *October* 1964)

The concept of labile nitrogenous matter in the body which may be mobilized during stress conditions and which has become variously known as protein stores, or reserves, is not new. According to Madden & Whipple (1940), in 1906 Morawitz demonstrated that, during starvation after severe depletion of plasma proteins through bleeding, plasma proteins were regenerated from what Madden & Whipple believed to be protein stores. Madden & Whipple (1940), who reported on a series of plasmaphaeresis experiments with dogs, concluded that plasma proteins formed 'part of a balanced system of body proteins' in which a 'steady state of ebb and flow exists between it [the plasma proteins] and a portion of the cell and tissue body proteins'. Yuile, Lucas, Neubecker, Cochrane & Whipple (1959) have since demonstrated that the drop in plasma proteins of dogs subjected to plasmaphaeresis was smaller than expected, because extravascular protein-building blocks found their way into the plasma proteins. From this observation one might conclude that extravascular proteins may serve as a source of reserve protein drawn upon for the synthesis of plasma proteins under conditions of stress. Fisher (1954) and Mitchell (1962) critically discuss the possible existence of reserve proteins. Both accept the presence in the body of a protein fraction which is readily katabolized when the animal is placed on a diet devoid of nitrogen or is subjected to certain stress conditions. The study of Longenecker & Hause (1958) may be interpreted as suggesting that free amino acids may also serve as protein reserves by making good dietary protein inadequacies.

The N excretion of animals on a protein-free diet generally displays a particular pattern. Initially the rate of excretion is rapid, but it gradually declines and becomes constant at a value usually referred to as the endogenous N excretion (Folin, 1905). According to Allison & Wannemacher (1957), the magnitude of reduction in N excretion to this constant value may be considered an estimate of protein reserves.

Shapiro & Fisher (1962) have reported the partial repletion of body N stores by non-essential amino acid N in adult cocks depleted to the endogenous level of N excretion. The non-essential amino acids were added to the repletion diet which provided the essential amino acids at levels just adequate for maintenance (Leveille & Fisher, 1958). Shapiro & Fisher suggested that 'the labile nature of protein reserves

* Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey. Supported in part by Grants-in-Aid from the National Science Foundation and US Public Health A-4904.

in conjunction with the dynamic state of certain body proteins permit metabolic shifts (namely de- and transamination) to take place at a rate sufficient to provide those amino acids, peptides or proteins necessary to maintain the protein reserves of the animal'. Apparently the cocks receiving the supplementary non-essential amino acid N were drawing on the same body N sources to supplement the ingested non-essential N; they thus became 'repleted' or gained N, as did Madden & Whipple's (1940) dogs in regenerating plasma proteins. The fact that cocks depleted to their endogenous level of N excretion did not require essential amino acids for partial repletion suggested a possible new approach to the estimation of the magnitude of protein stores. It was reasoned that the evident lack of requirement for supplementary essential amino acids might indicate that essential amino acids present in a reserve pool were available to supplement the dietary non-essential amino acids and thus allow a certain degree of repletion. The size of this reserve might well be estimated from the body N losses incurred up to the point at which non-essential amino acid N no longer will, but balanced proteins would, allow repletion.

The experiments now described were designed to study protein depletion and repletion in adult cocks, with special emphasis on the estimation of the magnitude of their 'protein reserves'. The N content of various body components was also determined.

EXPERIMENTAL

Studies on site of N loss

The purpose of this study was to examine the N content of various body components during protein depletion.

Plan. Adult, White Leghorn cocks between 1·5 and 2·5 years old were housed in individual cages. They were given a protein-free diet (for composition see Table 1, Wessels & Fisher, 1965) and their N excretion was measured continuously.

Table 1. *Mean values with their standard errors for body-weight and nitrogen excretion of White Leghorn cocks depleted of body N with a protein-free diet*

Depletion period (days)	Body-weight: Initial (wet) (g)	Body-weight: Final (wet) (g)	Body-weight: Final (dry, fat-free) (g)	N excreted during depletion period (mg)	Percentage body N loss
0	2503 ± 104	2503 ± 104	519·7 ± 14·6	0	0
2	2657 ± 60	2593 ± 67	557·9 ± 17·9	454 ± 78	0·530 ± 0·108
4	2536 ± 169	2383 ± 177	496·7 ± 39·8	2382 ± 261	2·991 ± 0·268
10	2700 ± 204	2290 ± 159	500·8 ± 43·0	5961 ± 770	6·670 ± 0·799

Groups of four cocks were killed after 0, 2, 4 and 10 days on the protein-free diet. Body-weights and the amount of N excreted by the cocks up to the time they were killed appear in Table 1. The birds were starved for 18 h and then killed by cutting the jugular veins in the pharynx. Blood was collected to obtain a measure of total plasma volume.

Analytical procedure. Plasma protein content was determined by the biuret method

as adapted for use with the AutoAnalyzer (Failing, Buckley & Zak, 1960). Lipid was extracted from weighed, oven-dried, body components by shaking the whole tissue or organ for at least 24 h in a 2:1 (v/v) mixture of chloroform–methanol. The solvent mixture was removed by filtration and the dried residue weighed. The lipid content was then obtained by difference. The N content of this lipid-free residue was determined by Kjeldahl digestion, followed by the colorimetric determination on the Auto-Analyzer (Ferrari, 1960) of ammonium ion in the digest with sodium phenate and sodium hypochlorite. The feathers and carcasses remaining after removal of the body components already analysed were dried in a forced-draught oven at 32° and ground. Lipid was removed as just described and portions of the lipid-free material were then analysed for N, as described for the body components.

Depletion–repletion studies

Eleven experiments, involving seventy-eight cocks and utilizing nine different N sources during repletion, were carried out.

Plan. Adult, White Leghorn cocks were used in all experiments. They were housed in individual metabolism cages in a temperature-regulated room (21°). Before the beginning of the experiment they had *ad lib.* access to the standard 15% protein diet used as the stock diet in our laboratory. Its percentage composition was: oatmeal 10·0, maize meal 36·5, wheat middlings 10·0, wheatmeal 10·0, soya-bean meal 15·0, meat meal 5·0, lucerne 3·0, limestone 6·3, trace-mineral mixture 0·1, dicalcium phosphate 1·2, dried maize distillers solubles 2·5, sodium chloride 0·4 and vitamins. Some of the birds had been given an exact amount of N from a similar but semi-refined diet, which contained soya-bean meal supplemented with methionine as the main N source, to provide 280 mg N/kg body-weight daily for 2–7 days before the beginning of the experiment. There were no indications that this pretreatment had any effect on subsequent N losses or repletion.

The animals were depleted to different degrees of body N loss and were, thereafter, placed on different repletion regimens for a 16-day repletion period. A complete record of N intake and excretion was kept for the duration of both the depletion and repletion periods. The manner of depletion was varied so that birds with different losses of body N could be obtained. The cocks consumed *ad lib.* a protein-free diet for 6–10 days. Those that were given the protein-free diet for less than 10 days were either starved for an additional 4–7 days or given a maintenance diet which supplied, in the form of free amino acids, an amount of dietary N equivalent to their endogenous N excretion (150 mg/kg body-weight daily, Leveille & Fisher, 1958).

During the 16-day repletion period the cocks were given diets supplying daily 280 mg N/kg body-weight, an amount previously shown to be adequate for the maintenance of non-depleted adult cocks (Leveille & Fisher, 1958) or for the repletion of depleted ones (Shapiro & Fisher, 1962). The N content of a diet containing zein was higher for reasons explained below (see p. 60). When all the N-containing diet had been consumed, a protein-free diet was made available at the rate of 15 g/kg body-weight daily.

During five of the last six trials blood for chemical analysis was drawn from the

wing veins of the cocks. Predepletion blood samples were obtained from twelve of these cocks and prerepletion samples from an additional seven. All blood was taken immediately before food was offered, i.e. after about 18 h of fasting.

Twenty-two depleted cocks were killed at the end of the trials.

Diets. The diet used in some instances to maintain cocks at their endogenous level of N excretion during the depletion period supplied 150 mg N, and all the repletion diets supplied 280 mg N/kg body-weight daily. These diets were given at the daily rate of 25 g/kg of the body-weight attained by the cocks at the beginning of the depletion or repletion periods respectively. In both types of diet a mixture of essential amino acids provided 115 mg N/kg body-weight daily which was supplemented either with 35 mg N/kg body-weight daily (used during the depletion period) provided by a mixture of L-glutamic acid and glycine to give a total of 150 mg N/kg body-weight daily, or with various protein and amino acid sources to supply 165 mg or a total of 280 mg N/kg body-weight daily for the repletion diets. The compositions of the protein-free diet and of the mixture of essential amino acids which supplied the 115 mg N/kg body-weight daily were identical with those described by Shapiro & Fisher (1962). The following protein sources were used in different repletion diets: egg albumin, 12·19% N; gelatin, 13·55% N; extracted soya-bean protein, 12·41% N; zein, 13·71% N; menhaden fish meal, 9·44% N. The amino acids and amino acid mixtures studied in repletion diets included: aspartic acid, equal quantities by weight of glycine and glutamic acid, a mixture simulating the amino acid composition of whole-egg protein, and a mixture in which half the N was supplied by amino acids mixed to simulate whole-egg protein and the other half by equal quantities of glycine and glutamic acid. Since we found (with rats, unpublished results) an apparent digestibility coefficient of 49% for zein, twice the amount of supplementary zein N (330 mg instead of 165 mg/kg body-weight daily) was included in the repletion diet containing zein.

All diets were provided in pelleted form.

Analytical procedure. The N content of the diets was determined in the same way as that of the body components (see p. 59). The excreta collected on alternate days were homogenized with water and then analysed for N in the same way as previously described.

The blood drawn from the wing veins was heparinized and centrifuged in graduated centrifuge tubes and the haematocrit determined from the total blood and packed cell volumes; subsequently total plasma N was determined in the same manner as for body components and food. Plasma protein content was determined by the procedure already described (see p. 58), and amino N content was estimated by Danielson's method as described by Hawk, Oser & Summerson (1954). Plasma creatine plus creatinine levels were determined on plasma obtained at the end of the last two experiments only, by the method of Owen, Iggo, Scandrett & Stewart (1954), after hydrolysis with 2 parts N-sulphuric acid for 45 min under 15 lb pressure in an autoclave.

The lipid content of the carcasses was again determined by difference after removal of the lipid with a 2:1 (v/v) chloroform–methanol mixture; body N was determined as

described before. Since our present studies on site of N loss indicated that the liver lost a greater percentage of its N on depletion than the other body components, certain liver analyses were carried out on the cocks that were killed. Immediately after killing, livers of the cocks were removed and a preliminary homogenate was prepared with ice in a Waring blendor. Nucleic acids (RNA and DNA) were determined on weighed portions of this material, which was finely homogenized with 95% ethanol in a tissue grinder. The procedure used for the nucleic acid determinations was a modified Schneider method as described by Mendes & Waterlow (1958). N analysis of the

Table 2. *Mean nitrogen values with their standard errors for body components of groups of four White Leghorn cocks depleted of body N on a protein-free diet*

Measurement	Cocks depleted for (days) 0	2	4	10
Blood				
Volume (% fat- and feather-free body-weight)	0·224 ± 0·009	0·248 ± 0·018	0·232 ± 0·013	0·197 ± 0·018
Haematocrit (%)	46·6 ± 2·24	41·9 ± 2·08	42·4 ± 1·52	44·9 ± 2·41
Plasma proteins:				
g/100 ml	4·564 ± 0·317	4·438 ± 0·393	3·324 ± 0·301	4·461 ± 0·268
% fat- and feather-free body-weight	0·547 ± 0·011	0·596 ± 0·042	0·422 ± 0·039	0·479 ± 0·036
Organs (% fat- and feather-free body-weight)				
Liver	0·187 ± 0·003	0·174 ± 0·003	0·165 ± 0·017	0·144 ± 0·010
Muscle (m. pectoralis)	0·535 ± 0·024	0·531 ± 0·062	0·521 ± 0·047	0·460 ± 0·043
Spleen	0·015 ± 0·001	0·013 ± 0·002	0·014 ± 0·001	0·012 ± 0·002
Gizzard	0·161 ± 0·011	0·190 ± 0·014	0·145 ± 0·014	0·142 ± 0·008
Bone (femur)	0·104 ± 0·004	0·099 ± 0·001	0·098 ± 0·003	0·095 ± 0·001
Heart	0·051 ± 0·003	—	0·065 ± 0·003	0·047 ± 0·005
Kidney	0·052 ± 0·003	0·052 ± 0·002	0·056 ± 0·004	0·049 ± 0·002
Small intestines	0·080 ± 0·005*	0·090 ± 0·017*	0·081 ± 0·012	0·080 ± 0·006
Feathers	3·95 ± 0·211	3·78 ± 0·120*	3·58 ± 0·027*	5·35 ± 0·306
Remainder feather-free carcass	10·62 ± 0·19	10·71 ± 0·31	11·01 ± 0·15	11·06 ± 0·14

Values after 2, 4 and 10 days of depletion expressed as a percentage of the 0-day value

Blood			
Volume (% fat- and feather-free body-weight)	111	104	88
Haematocrit (%)	90	91	96
Plasma proteins:			
g/100 ml	97	73	98
% fat- and feather-free body-weight	109	77	88
Organs (% fat- and feather-free body-weight)			
Liver	93	88	77
Muscle (m. pectoralis)	98	96	84
Spleen	90	97	79
Gizzard	118	90	88
Bone (femur)	95	94	91
Heart	—	126	92
Kidney	100	108	94
Small intestines	112	102	100
Feathers	96	91	125
Remainder feather-free carcass	101	104	104

* Only three organs were available for analysis.

liver homogenate was performed by colorimetric analysis on the AutoAnalyzer. Protein content of blood plasma and of liver homogenate, prepared in 10 ml water in a Ten Broeck tissue grinder (available from Arthur H. Thomas Co., Philadelphia, Pa), was determined by the biuret method as described earlier. The amino N content of liver homogenate was determined by the method previously described for plasma.

RESULTS

Studies on site of N loss

In order to avoid a masking effect due to differences in fat content of tissues, the N values were expressed as a proportion of the fat-free weight of the carcass devoid of feathers.

Haematocrit values dropped during initial depletion but increased again thereafter (after the 4th day). The plasma protein concentration displayed a similar pattern. The drop in blood volume with concurrent concentration of the plasma may explain the upward trend in haematocrit and plasma protein values with continued depletion.

During depletion (Table 2), liver, muscle, spleen and gizzard showed the greatest relative N loss (expressed as a percentage of fat-free body-weight). When expressed in the same manner as for other organs, the femur showed an early decrease only in N content. Initially the kidney and heart appeared to lose N at a slower rate than the rest of the carcass, as evidenced by the higher values observed for the 2- and 4-day depleted cocks.

In agreement with the findings of Summers & Fisher (1962), our results also showed a significant increase in relative and total feather N by the end of the depletion period. This increase was due to a marked loss in fat from the feathers.

Depletion–repletion studies

The correlation between amount of N lost per kg body-weight and percentage of the lost N regained on repletion was calculated for those N supplements with which repletion was carried out from seven or more stages of prior depletion. The resultant correlation coefficients appear on the last line of Table 3. Regression equations were calculated for aspartic acid, gelatin and fish meal as repletion supplements, since for these additives the correlation coefficients were statistically significant and a sufficient number of values was available. The regression equations were:

Aspartic acid	$Y = 104{\cdot}88 - 40{\cdot}8X$	$(n = 22)$,
Gelatin	$Y = 146{\cdot}22 - 45{\cdot}9X$	$(n = 13)$,
Fish meal	$Y = 178{\cdot}11 - 44{\cdot}8X$	$(n = 10)$,

where Y represents repletion as a percentage of the N depletion loss, X represents the body N lost during depletion and n refers to the number of animals involved. Fig. 1 shows the observations plotted about the fitted regression lines. The intercept values (N loss) of the regression lines with the zero retention line (Fig. 1) have been converted from mg N loss/kg body-weight into a percentage of total body N. For this conversion an average body N value of 37·3 g/kg body-weight, based on carcass analyses of twenty-two depleted cocks, was used. The intercept values as a percentage

of total body N were 6·9 and 8·6 for, respectively, aspartic acid and gelatin. An intercept value for fish meal seems unrealistic, since presumably such a good-quality protein would permit repletion from any state of protein depletion.

Table 3. *Nitrogen retention expressed as a percentage of N loss of depleted White Leghorn cocks given different dietary N supplements*†

N loss		Aspartic acid		Glutamic acid plus glycine		Gelatin		Isolated soya protein	
g/kg body-wt	% of body N	No. of cocks	N retained (%)	No. of cocks	N retained (%)	No. of cocks	N retained (%)	No. of cocks	N retained (%)
< 1·4	< 3·75	3	47·3	1	40·5	4	80·7	—	—
1·4–1·7	3·8–4·6	5	51·7	4	6·2	1	72·9	3	70·3
1·7–2·0	4·6–5·4	4	38·7	—	—	—	—	1	63·1
2·0–2·3	5·4–6·2	4	5·6	1	10·6	2	47·1	—	—
2·3–2·6	6·2–7·0	4	−8·8	1	5·1	2	45·4	2	31·5
2·6–2·9	7·0–7·8	1	−7·3	—	—	1	−10·9	1	3·3
2·9–3·2	7·8–8·6	1	17·2	1	−0·7	1	4·8	—	—
3·2–3·5	8·6–9·4	—	—	—	—	2	−8·0	—	—
> 3·5	> 9·4	—	—	—	—	—	—	—	—
Correlation coefficients ¶		−0·758**		−0·486		−0·818**		−0·837*	

N loss		Fish meal		Egg-simulated amino acid mixture		Diluted egg-simulated amino acid mixture‡		Albumin or zein	
g/kg body-wt	% of body N	No. of cocks	N retained (%)	No. of cocks	N retained (%)	No. of cocks	N retained (%)	No. of cocks	N retained (%)
< 1·4	< 3·75	1	135·8	—	—	—	—	2	75·6§
1·4–1·7	3·8–4·6	1	109·9	1	36·8	—	—	3	64·8§
1·7–2·0	4·6–5·4	—	—	2	26·9	—	—	—	—
2·0–2·3	5·4–6·2	1	66·6	3	20·3	—	—	—	—
2·3–2·6	6·2–7·0	3	58·5	1	18·6	—	—	—	—
2·6–2·9	7·0–7·8	1	79·4	1	3·8	—	—	1	32·9‖
2·9–3·2	7·8–8·6	2	42·6	—	—	2	−5·4	—	—
3·2–3·5	8·6–9·4	—	—	—	—	1	−6·9	—	—
> 3·5	> 9·4	1	27·0	—	—	1	−11·2	—	—
Correlation coefficients ¶		−0·901**		−0·904**					

* Significant at $P < 0.05$; ** significant at $P < 0.01$.

† Added to the repletion diets in the quantities and manner indicated on p. 60.

‡ Half the N was supplied by an amino acid mixture simulating whole-egg protein and the rest by glutamic acid and glycine.

§ Albumin was used as N supplement.

‖ Zein was used as N supplement.

¶ Relationship between body N loss during depletion and percentage retention during repletion.

It is clear from the regression lines of Fig. 1 and the detailed results presented in Table 3 that the degree of N repletion during the 16-day periods was linearly influenced by the degree of prior depletion. Severely depleted cocks retained less or failed to retain any N, depending upon the N supplement, whereas all moderately depleted birds retained N, irrespective of the N source.

In the comparison of the efficiency of different N supplements for repletion, the

results indicate that the proteins usually considered as of good quality were, in allowing N retention at any given level of N depletion, on the whole, superior to the non-essential amino acids or the poorer-quality proteins.

Aspartic acid was effective in permitting repletion when the body N loss did not exceed 2·6 g/kg body-weight (6·9%). Gelatin, even though totally devoid of an essential amino acid (tryptophan), permitted N retention at body N depletion up to 3·2 g/kg body-weight (8·6%). The high-quality protein (fish meal) permitted excellent repletion over the entire range of N depletion studied.

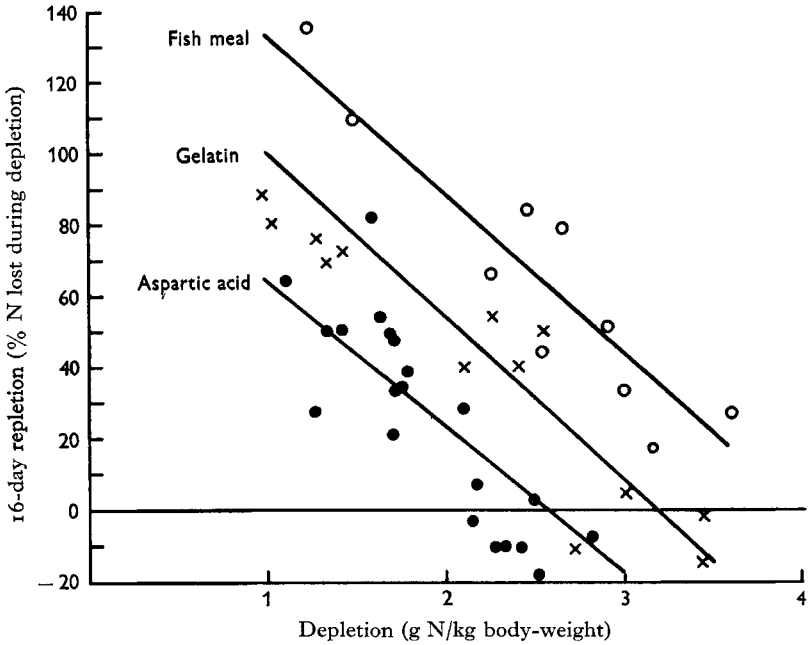

Fig. 1. Nitrogen repletion as a function of prior N depletion in adult cocks fed on a maintenance mixture of amino acids and given aspartic acid, gelatin, or fish meal as the source of supplementary N. ○, fish meal ($Y = 178{\cdot}1-44{\cdot}8X$; $n = 10$); ×, gelatin ($Y = 146{\cdot}7-45{\cdot}9X$; $n = 13$); ●, aspartic acid ($Y = 104{\cdot}9-40{\cdot}8X$; $n = 22$).

Of the other N supplements used for repletion, the glutamic acid and glycine mixture was utilized similarly to aspartic acid. A most interesting observation was recorded with the mixture in which half the N was supplied by amino acids simulating whole-egg protein and half by glycine and glutamic acid. This N supplement was ineffective in permitting N retention in cocks depleted to levels similar to those from which some repletion still occurred when gelatin was used as the supplement.

The blood analysis values appear in Table 4. For the depletion phase of the study, values are given in absolute units, whereas the repletion values are presented in terms of correlation coefficients that show the relationship between percentage repletion experienced, and either changes in blood composition during the repletion period or blood composition values recorded at the completion of the 16-day repletion period. During depletion, haematocrit values, total plasma N and plasma protein concentrations decreased significantly. Upon repletion, only creatine plus creatinine showed

a significant change (decrease). A comparison, not shown in Table 4, was made between cocks that did and those that did not retain N on various repletion diets. The former group had significantly less ($P < 0.01$) plasma creatine plus creatinine

Table 4. *Blood nitrogen constituents for groups of nine to nineteen White Leghorn cocks before and after protein depletion*

	Before depletion	After depletion	Difference	Correlation coefficients with % body N repletion and: % change in blood composition during repletion	Correlation coefficients with % body N repletion and: Blood composition at end of repletion period†
Haematocrit (%)	48·90	45·48	3·42*	−0·364	0·194
Plasma N (g/100 ml)	0·71	0·61	0·10*	0·656	0·213
Plasma protein (g/100 ml)	4·594	3·599	0·995**	0·426	0·213
Plasma amino N (mg/100 ml)	13·1	11·2	1·9	−0·259	0·163
Creatine + creatinine (mg/100 ml)	—	2·364	—	—	−0·798**

* Significant at the 5 % test level; ** significant at the 1 % test level.

† Calculated from forty-nine analyses for plasma N, forty-three for plasma proteins, fifty-three for plasma amino N and twenty-six for creatine plus creatinine.

Table 5. *Liver nitrogen components of White Leghorn cocks after protein depletion and repletion*

	After depletion†	After repletion*†: Effective	After repletion*†: Ineffective
Liver N:			
As % of fat-free body-weight	0·1233 ± 0·0093 (7)	0·1069 ± 0·0087 (10)	0·1069 ± 0·0072 (7)
In relation to liver-DNA extinction units	3·42 (2)	3·22 ± 0·153 (10)	2·70 ± 0·243 (7)
Liver proteins:			
As % of fat-free body-weight	0·1398 ± 0·0111 (7)	0·1153 ± 0·0132 (10)	0·1081 ± 0·0088 (7)
In relation to liver-DNA extinction units	2·96 (2)	3·86 ± 0·62 (10)	2·78 ± 0·24 (7)
Liver amino nitrogen:			
As % of fat-free body-weight × 10	0·1073 ± 0·0061 (7)	0·0635 ± 0·0077 (10)	0·0656 ± 0·0078 (7)
In relation to liver-DNA extinction units	2·04 (2)	1·91 ± 0·17 (10)	1·62 ± 0·09 (7)
Liver RNA (extinction units):			
As % of fat-free body-weight	1·483 ± 0·198 (3)	0·584 ± 0·042 (10)	0·723 ± 0·058 (7)
In relation to liver-DNA extinction units	1·34 (2)	1·666 ± 0·188 (10)	1·576 ± 0·242 (7)

Values are significantly different ($P < 0.05$) except when they share a common underlining; the 'after depletion' values expressed in relation to liver DNA are excluded from these calculations.

* At the end of the 16-day period during which repletion diets were given; 'Effective' refers to cocks that retained some N during the repletion period (ten out of seventeen); 'Ineffective' refers to those cocks (seven out of seventeen) that failed to retain N.

† Means with standard errors; figures in parentheses are the numbers of livers analysed.

(20·64 μg/ml) than the latter group (28·691 μg/ml). Some of the r values, including those for haemotocrit, plasma N, and plasma protein, approached significance at the 10% level of probability.

Liver composition values are given in Table 5 for cocks killed after depletion or after a period of repletion. The values are presented on the basis of (*a*) fat-free body-weight and (*b*) liver DNA. The DNA values are given as extinction readings. Only ten of the seventeen cocks killed after repletion retained N (column marked 'Effective', Table 5) while seven failed to retain N (column marked 'Ineffective', Table 5). Of the liver N components analysed, when expressed as a percentage of fat-free body weight, amino N and RNA showed a significant decrease whether or not repletion was effective. The liver protein value for the ineffectively repleted cocks, but not that for the effectively repleted ones, was also significantly lower than that for depleted cocks.

When liver N components were expressed on a DNA basis, the ineffectively repleted cocks tended to display lower values than did the depleted or effectively repleted cocks. This observation suggests a certain degree of recovery on diets permitting N retention.

DISCUSSION

Body composition changes during depletion and repletion

We did not observe in adult cocks the considerable N loss from the alimentary tract reported by Addis, Poo & Lew (1936) for adult rats starved for 7 days. The reason for this apparent discrepancy may be explained on the basis of the continued intestinal activity in the cocks associated with the eating of the protein-free diet. This contention is supported by the work of Luck (1936) who showed no intestinal N loss in adult rats on a low-protein diet. Ju & Nasset (1959), on the other hand, killed rats after 8 days on a protein-free diet and showed a 24% N loss from the small intestines and an equal amount from the stomach. Addis *et al.* (1936) observed considerable N loss from liver, heart, and a composite of muscle, skin and skeleton in rats on a protein-free diet. Dickerson & McCance (1960) observed a considerable loss in protein N from the pectoralis muscle of 27-week-old cockerels whose diet had been restricted in amount for 15 weeks. With certain exceptions, such as the stability of kidney and heart N of the adult cocks, our depletion results are in general agreement with those cited. The relatively small extent of liver protein repletion was not unexpected, since for the majority of the animals in this study the degree of repletion was less than two-thirds the N lost during depletion. Since the liver is considered a major site for deposition of 'protein reserves', and since, in absolute terms, most of the depletion N loss occurs in muscle, the liver may be one of the last organs to show the effects of repletion.

In their work with Jamaican infants, Waterlow & Weisz (1956) reported results that are similar to those observed in the present study. When their infants were recovering from protein malnutrition, they had less liver RNA than upon admission to the hospital. In our study there was only a small increase in RNA during repletion (Table 5).

Repletion with different N sources

The presence in the adult animal of amino acid, peptide, or protein pools which may be called upon to complement an intake of dietary non-essential amino acids and thus allow the animal, if not too severely depleted, to retain N is demonstrated by this study. This corroborates the findings reported earlier by Shapiro & Fisher (1962). Observations similar to those herein reported for adult cocks have been found also for adult man. Fisher, Brush, Shapiro, Wessels, Berdanier, Griminger & Sostman (1963) demonstrated a significant N retention from an imbalanced, high-protein diet, deficient in tryptophan, in male college students who had previously experienced a period of negative N balance on a low-protein diet inadequate in tryptophan. The subjects did not retain N, when the giving of the imbalanced diet was preceded by a period of N retention or equilibrium on an adequate, balanced-protein intake.

The greater success of the poor-quality protein (gelatin) compared with that of aspartic acid, in repleting depleted cocks was undoubtedly due to the greater variety of amino acids contributed by this N source. Thus, cocks given aspartic acid retained N if the prior depletion loss did not exceed 6·9% of body N. Gelatin was effective up to a body N loss of 8·5%. These results also support the suggestion by Mitchell (1962) that the adult subject has a 'particulate' amino acid requirement, allowing for the utilization of individual dietary amino acids even in the complete absence of other essential amino acids from the diet. He contrasts this with the 'aggregate' amino acid requirement typical of the growing animal which must have all needed amino acids present in its diet simultaneously.

The repletion results with the N supplement in which half, or 83 mg N/kg body-weight daily, was derived from an amino acid mixture simulating the composition of whole-egg protein are particularly noteworthy. This supplement was given to cocks that had lost in excess of 6·9% body N (depleted beyond protein reserves). We believe that this supplement proved ineffective because the ratio of essential to non-essential amino acids was far out of line, the importance of which has been emphasized by the thorough studies of Swenseid (1963) (mis-spelling of Swendseid in article quoted).

If we are correct in assuming that the effectiveness of aspartic acid (or glutamic acid plus glycine) in permitting N repletion is related to the protein reserves of the body, it follows that this study provides a quantitative measure of this reserve pool. From this reasoning the protein reserves may be considered to have been depleted (perhaps of only one essential amino acid) when 2·6 g N/kg body-weight, or 6·9% body N, had been lost by the adult cocks.

A protein reserve of 6·9% of body N is a higher estimate than that of Madden & Whipple (1940) which was based on studies of plasma protein regeneration. Two of their dogs regenerated plasma protein to the extent of 7·56 g and 5·14 g/kg body-weight. Assuming that plasma protein contains 16% N, this regeneration is equivalent to 1·2 and 0·8 g N/kg body-weight, or half that estimated in the cocks. This difference, which may be due to the species studied, may also be due to other body proteins besides those of the plasma having been depleted and replaced during repletion in the study of Madden & Whipple.

It is interesting that the regression lines for aspartic acid, gelatin and fish meal (Fig. 1) relating percentage repletion to percentage body N loss during depletion had essentially the same slope (were parallel to one another). This observation suggests that the ratios of the *Y* intercept values may be useful as a measure of the repletion potential of dietary N sources.

Attention is drawn to the large deviation from expectation by certain cocks during repletion. The N loss per kg body-weight of different animals cannot be regarded as an accurate measure for comparing percentage body N loss. Large differences in body composition (Summers & Fisher, 1962), which prevent an accurate estimate of body N content, may account for the deviations from expected repletion rates.

SUMMARY

1. Body components of sixteen adult cocks given a protein-free diet for 0, 2, 4, or 10 days were compared with respect to nitrogen content; the livers of twenty-three cocks, seven of which had been similarly protein-depleted for 10 days and the other sixteen either partly repleted or unable to retain the dietary N offered, were also compared. Liver lost the greatest percentage of its N but, in terms of total body N loss, muscle and probably bone lost most N. The livers from repleted cocks had a higher total N content than those from cocks that did not retain N during the repletion period (on a liver DNA basis).

2. Adult White Leghorn cocks (including the twenty-three on which liver analyses were performed) were depleted of different amounts of body N during 10–13 days, during most of which time they received a protein-free diet. Their depletion was followed by 16 days of repletion with different N sources added to an amount of essential amino acid N just sufficient to maintain the cocks' endogenous N excretion.

3. The greater the degree of body N depletion, the smaller was the extent of repletion obtained during the 16-day repletion period with all N sources. Aspartic acid, as the sole N supplement, allowed repletion of body N provided not more than 6·9% body N had been lost. Other N sources, including gelatin and especially fish meal, were effective in repleting more severely depleted birds.

4. The effectiveness of aspartic acid to replete body N losses is discussed in terms of labile N components and protein reserves of the body. The amount of body N loss beyond which repletion did not occur with a non-essential amino acid supplement was regarded as a measure of the size of the protein reserve pool.

We would like to express our appreciation to Dr Ralph Shapiro for his stimulating discussions and critical review of this manuscript.

REFERENCES

Addis, T., Poo, L. J. & Lew, W. (1936). *J. biol. Chem.* **115**, 111.
Allison, J. B. & Wannemacher, R. W. Jr. (1957). *Amino Acid Malnutrition.* New Brunswick, New Jersey: Rutgers University Press.
Dickerson, J. W. T. & McCance, R. A. (1960). *Brit. J. Nutr.* **14**, 331.
Failing, J. F., Buckley, M. W. & Zak, B. (1960). *Amer. J. clin. Path.* **33**, 83.
Ferrari, A. (1960). *Ann. N.Y. Acad. Sci.* **87**, 792.
Fisher, H., Brush, M. K., Shapiro, R., Wessels, J. P. H., Berdanier, C. D., Griminger, P. & Sostman, E. R. (1963). *J. Nutr.* **81**, 230.
Fisher, R. B. (1954). *Protein Metabolism.* London: Methuen and Co. Ltd.
Folin, O. (1905). *Amer. J. Physiol.* **13**, 117.
Hawk, P. B., Oser, B. L. & Summerson, W. H. (1954). *Practical Physiological Chemistry*, 13th ed. New York: The Blakiston Co. Inc.
Ju, J. S. & Nasset, E. S. (1959). *J. Nutr.* **68**, 633.
Leveille, G. A. & Fisher, H. (1958). *J. Nutr.* **66**, 441.
Longenecker, J. B. & Hause, N. L. (1958). *Nature, Lond.*, **182**, 1739.
Luck, J. M. (1936). *J. biol. Chem.* **115**, 491.
Madden, S. C. & Whipple, G. H. (1940). *Physiol. Rev.* **20**, 194.
Mendes, C. B. & Waterlow, J. C. (1958). *Brit. J. Nutr.* **12**, 74.
Mitchell, H. H. (1962). *Comparative Nutrition of Man and Domestic Animals.* Vol. 1. New York and London: Academic Press Inc.
Owen, J. A., Iggo, B., Scandrett, F. J. & Stewart, C. P. (1954). *Biochem. J.* **58**, 426.
Shapiro, R. & Fisher, H. (1962). *J. Nutr.* **76**, 106.
Summers, J. D. & Fisher, H. (1962). *Z. Ernährungsw.* **3**, 48.
Swenseid, M. E. (1963). *In Proceeding of Symposium on Protein Nutrition and Metabolism. Spec. Publ. Ill. agric. Exp. Sta.* no. 4, p. 37.
Waterlow, J. C. & Weisz, T. (1956). *J. clin. Invest.* **35**, 346.
Wessels, J. P. H. & Fisher, H. (1965). *Brit. J. Nutr.* **19**, 71.
Yuile, C. L., Lucas, F. V., Neubecker, R. D., Cochrane, C. G. & Whipple, G. H. (1959). *J. exp. Med.* **109**, 165.

Printed in Great Britain