BLOOD CHEMISTRY AS AN INDICATOR OF NUTRITIONAL CONDITION IN BIGHORN SHEEP

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INTRODUCTION

Experimental animal physiology studies use basline values established with a control group to determine the significance of changes of a particular physiological parameter. Blood chemistry studies in wildlife biology have attempted to establish baseline values in relation to many uncontrolled variables and to utilize relative differences in values between seasons, habitat types and populations to assess animal condition and population status.

The last ten years have witnessed a dramatic increase in the use of blood chemistry to determine the taxonomic, clinical, physical, physiological and nutritional status of wild ungulates and carnivores (Cowan and Johnston 1962; Anderson et al. 1972; Cowan and Bandy 1969; Hebert 1972; Barrett and Chalmers 1977; Vaughen et al. 1973; Le Resche et al. 1974; Dieterich 1970; Kitts et al. 1956; Franzmann and Thorne 1970; Skeen 1974; Weber 1973; Pearson and Halloran 1972; Seal et al. 1975, 1978a, 1978b; Seal and Hoskinson 1978; Eubanks et al. 1976). There has been a gradual shift from assessment of carrying capacity by vegetation utilization studies and change in population characteristics to that of establishment of nutritional status of wild populations. Le Resche et al. (1974), suggests that the trend in research has been toward analyses of individual primary factors rather than of collective manifestations. In general, current research incorporates quantitative assessment of range and animal numbers with qualitative aspects of range (crude protein (CP), crude

fibre, gross energy (GE)) - animal (blood urea nitrogen (BUN), serum inorganic phosphorus, hematocrit) relationships.

Domestic animal literature has documented many of these relationships, especially the clear relationship between BUN and protein intake, at levels of protein intake above maintenance (Lewis 1957; McIntyre 1970; Preston et al. 1965; Somers 1961; Mukhoty et al. 1969).

By comparison, cholesterol baseline values have been collected for wild ungulates (Le Resche et al. 1974; Seal et al. 1978b; Weber 1973), but few studies have determined its relationship with nutritional or energy status. Although Le Resche et al. (1974), states that cholesterol level reflects diet, dietary change, the state of rumen metabolism and appears to indicate a state of malnutrition, it can be altered by sex, age, season and the level of saturated fatty acids consumed. Seal et al. (1978b) suggests that cholesterol was only moderately affected by dietary composition in white-tailed deer fawns. Similarly, few studies have utilized hemoglobin or hematocrit to assess general nutritional status between populations. Karns and Seal (from Le Resche et al. 1974), indicate that hemoglobin concentration in male Minnesota moose declined between October and December, and Kirk and Davis (1970) indicate a seasonal effect on hemoglobin and hematocrit in range cattle. Seal et al. (1972), found significantly lower hemoglobin and hematocrit levels in white-tailed deer between a moderate and high diet group. Ullrey et al. (1967) used isocaloric diets varying in crude protein from 8-20 per cent and found no differences in hemoglobin or hematocrit levels between groups, while Seal et al. (1978b) found similar hematocrit levels and higher hemoglobin levels for fawns on low protein diets between groups of

white-tailed deer fawns on low and high protein and energy diets.

The purpose of this paper is to explore the relationship between BUN, serum cholesterol, hematocrit and hemoglobin and a host of nutritional measurements conducted under controlled conditions, at or below maintenance.

ACKNOWLEDGEMENTS

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METHOD AND MATERIALS

Two groups of Rocky Mountain bighorn sheep were maintained on natural rangeland diets in order to simulate a migratory and non-migratory situation. The feeding regime and nutritional measurements have been described previously (Hebert 1972, 1973). Serum samples were collected monthly from September 1969 to May 1970 (Hebert 1972) and analyzed (Hebert 1972, Franzmann 1971) for an array of variables other than those reported (BUN, hematocrit, hemoglobin, cholesterol) in this paper. There were eight serum sampling periods but only two complete nutritional trials in the fall (1969) and two the following spring (1970). Scaled curves representing nutritional parameters were extrapolated between the last fall trial and the first spring trial in order to obtain an estimate of each nutritional parameter for each blood sampling period. Regression analysis was used to establish the relationship between serum constituents and

nutritional measurements. The initial relationship consisted of all sampling dates from October to May. The second computer run eliminated the last sampling date (high quality pelleted ration), while the third computer run eliminated the first and last date for both groups. The fourth and fifth computer run eliminated the fifth and last sampling date and the first, fifth and last sampling date, respectively. This reduction in sampling dates removed anabolic effects of the high quality pelleted diet fed in May and removed the effects of a multiple vitamin supplement administered in February. In addition, it allowed by difference comparisons of levels of significance due to the effects of changes in diet quality.

STUDY AREA

The nutritional and blood chemistry assessment were conducted in bighorn sheep holding facilities in the East Kootenay region of British Columbia (Hebert 1973).

RESULTS

BUN

Blood urea nitrogen values for a simulated migratory and non-migratory group of bighorn sheep changed from an average value of 14.6 mg/100 ml during early fall to 24.2 mg/100 ml in late winter (Figure 1). During the October 1 sampling period the migratory group was ingesting good quality alpine forage (CP 11.59%) which produced a comparable BUN value of 18.5 mg/100 ml. Conversely, the non-migratory group was ingesting a submaintenance diet (CP 2-3%) which produced a low BUN value (8.8 mg/100 ml). During the period October 1 to April 14 both groups subsisted on submaintenance diets containing 2-3% CP. These diets are reflected by

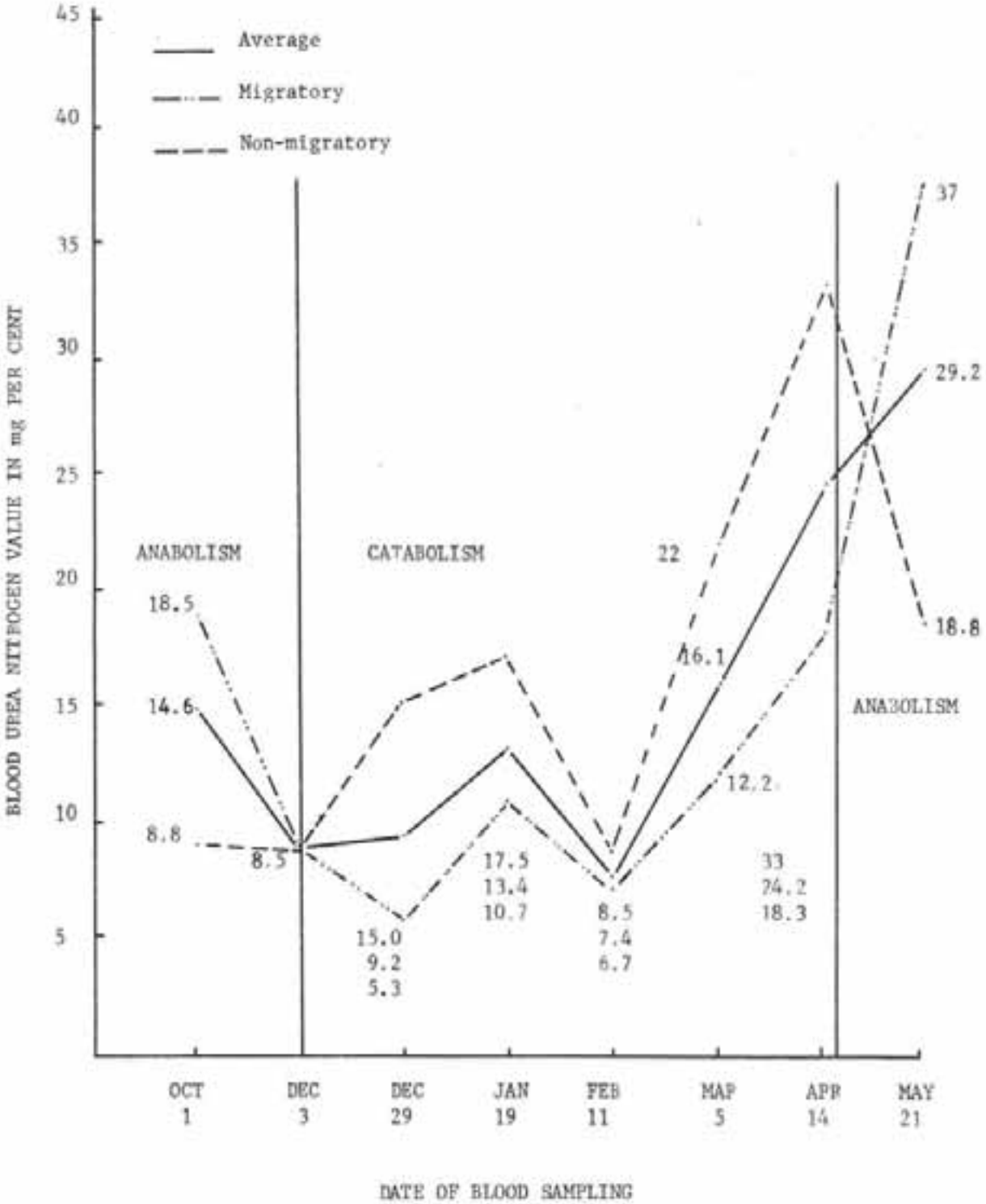

FIGURE 1.

The seasonal pattern of blood urea nitrogen, reflecting high and low quality diets.

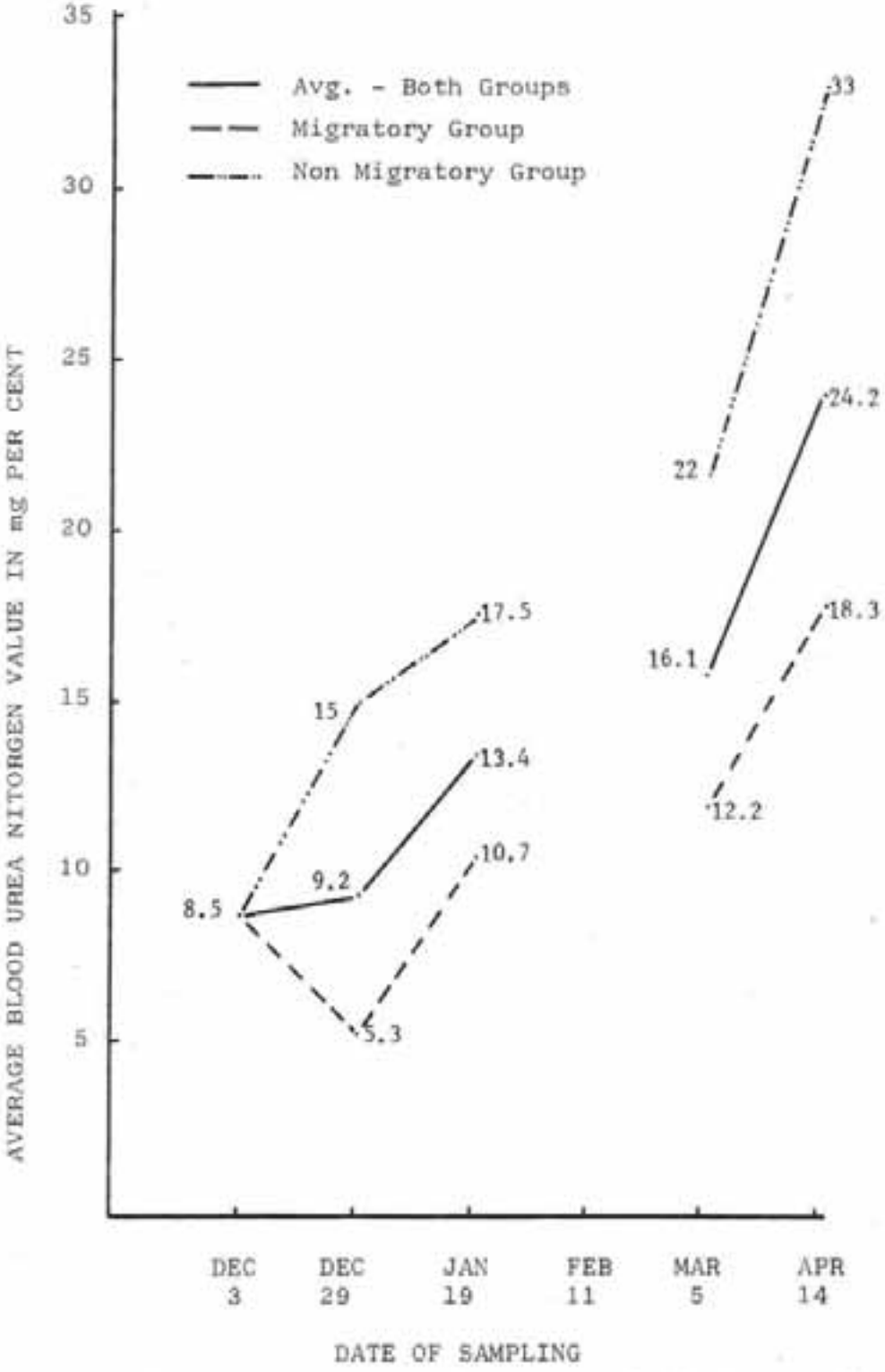

FIGURE 2 The seasonal pattern of blood urea nitrogen while on sub-maintenance diets and without the effects of multiple vitamin supplements (February 11).

an increase in the BUN value from 8.5 mg/100 ml. to 24.2 mg/100 ml as body catabolic processes provided nitrogen to the blood. Subsequently, the curves diverged during the spring period (May) as BUN became related to protein intake rather than tissue protein when both groups were placed on a high quality pelleted ration (21% CP) to simulate spring forage growth. The 5 week period between the April and May sampling dates caused the two groups to change at a different rate, from a catabolic state in the production of BUN to one related to CP intake. The migratory group, being in better body condition (Hebert 1973), adjusted to the improved diet more rapidly and increased its BUN level proportionately. The non-migratory group was in poorer body condition and the May sampling point appeared to indicate that the BUN level may not completely reflect the quality of the diet or that it was reflicting the generally poorer condition of this group when returned to a state of anabolism. During the February sampling period the animals were stressed for S-GOT value measurement and were on low quantities of a multiple vitamin which may have affected the BUN value. If this point is removed (Figure 2), the contribution of the body tissues to BUN values shows a continuous increase from December 29 - April 14 while the groups are on submaintenance diets.

HEMATOCRIT AND HEMOGLOBIN

During the sampling period, the migratory group (Figure 3) maintained a higher level of both hematocrit (average 48.3%) and hemoglobin (average 18.7%) than did the non-migratory group (38.5% + 13.9%, respectively). The data did not indicate seasonal trends for either variable but did suggest that previous nutritional differences did influence the level while the two groups were on submaintenance diets. Seal et al.

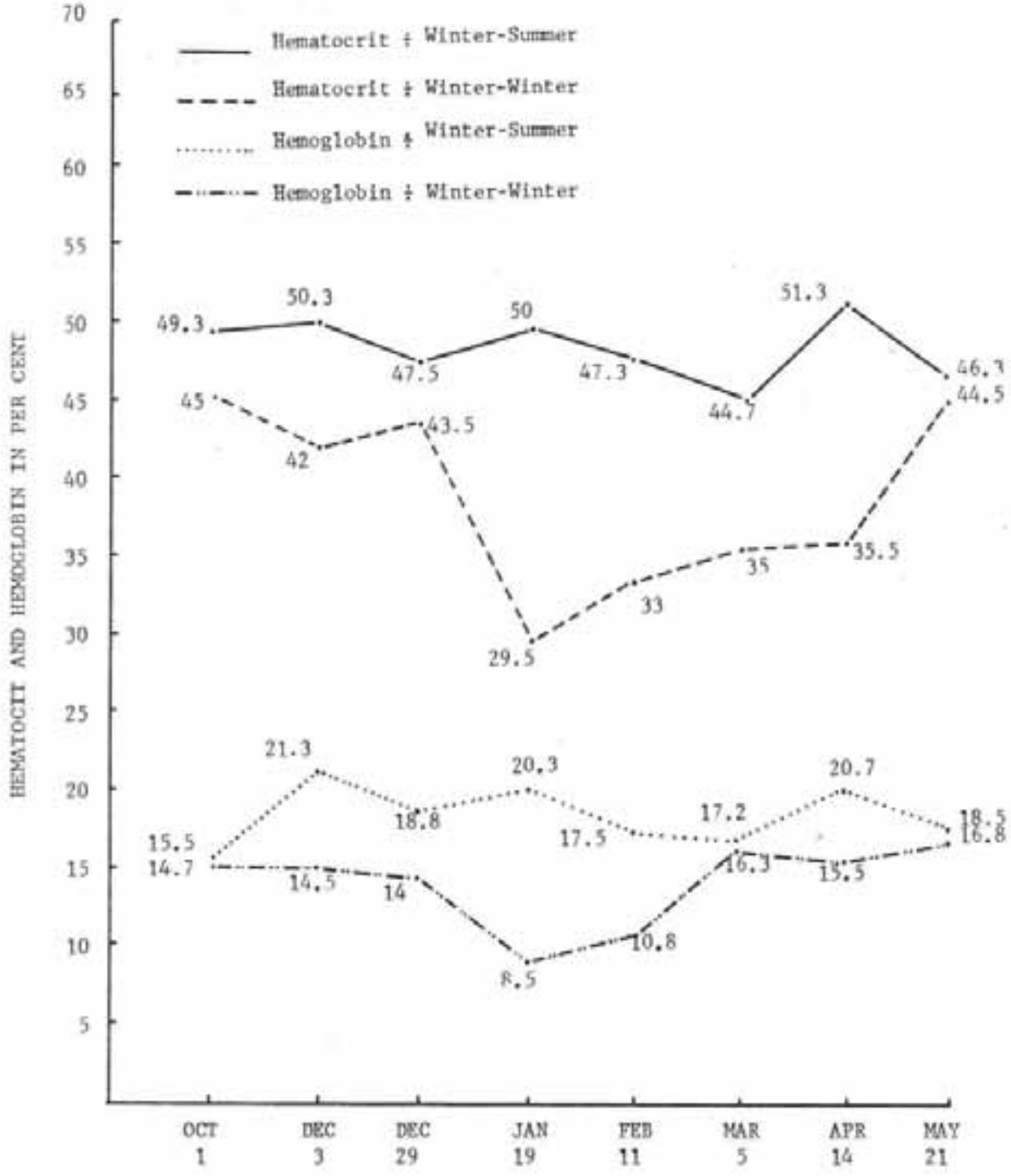

FIGURE 3

Seasonal changes in hematocrit and hemoglobin between two groups of sheep on high and low quality diets.

(1972) obtained similar results with deer. Similarly, Franzmann (1972) presented a highly significant (P = .0001) relationship between PCV and condition in his model, and the positive ranking of PCV means with condition classes in the Duncan's multiple range test suggest that PCV values are valid indicators of condition of bighorn sheep. Similarly, Meacham et al. (1964) found that both hematocrit and hemoglobin were significantly lower for protein deficient bulls. In contrast, Ullrey et al. (1967) used approximately isocaloric diets with crude protein contents of 8,13 and 20 per cent, but found no differences in hemoglobin or hematocrit levles between groups.

CHOLESTEROL

The change in cholesterol value does not appear to indicate any specific trend from fall to spring (Figure 4). However, the seasonal average for the migratory group was 86.6 mg/100 ml while that for the non-migratory group was only 72.5 mg/100 ml. The trend and the actual values indicated in this study (a peak in the fall, decline throughout the winter and an increase prior to spring green up) are very similar to that shown by Le Resche et al. (1974) for moose in Alaska. They suggest that lower values in Minnesota moose (49-56 mg/100 ml) may be attributable to differences in diet and rumen function. Serum cholesterol levels obtained in this study were higher than those obtained by Seal et al. (1978) for white-tailed deer fawns (41 - 60 mg/dl), Barrett and Chalmers (1977) for antelope (37 - 58 mg/dl) and similar to those obtained by Seal et al. (1978b) (67-90 mg/dl) in adult white-tailed deer. Franzmann (1972) obtained serum cholesterol values for bighorn sheep which varied from 71-96 mg/100 ml due to variations in excitability.

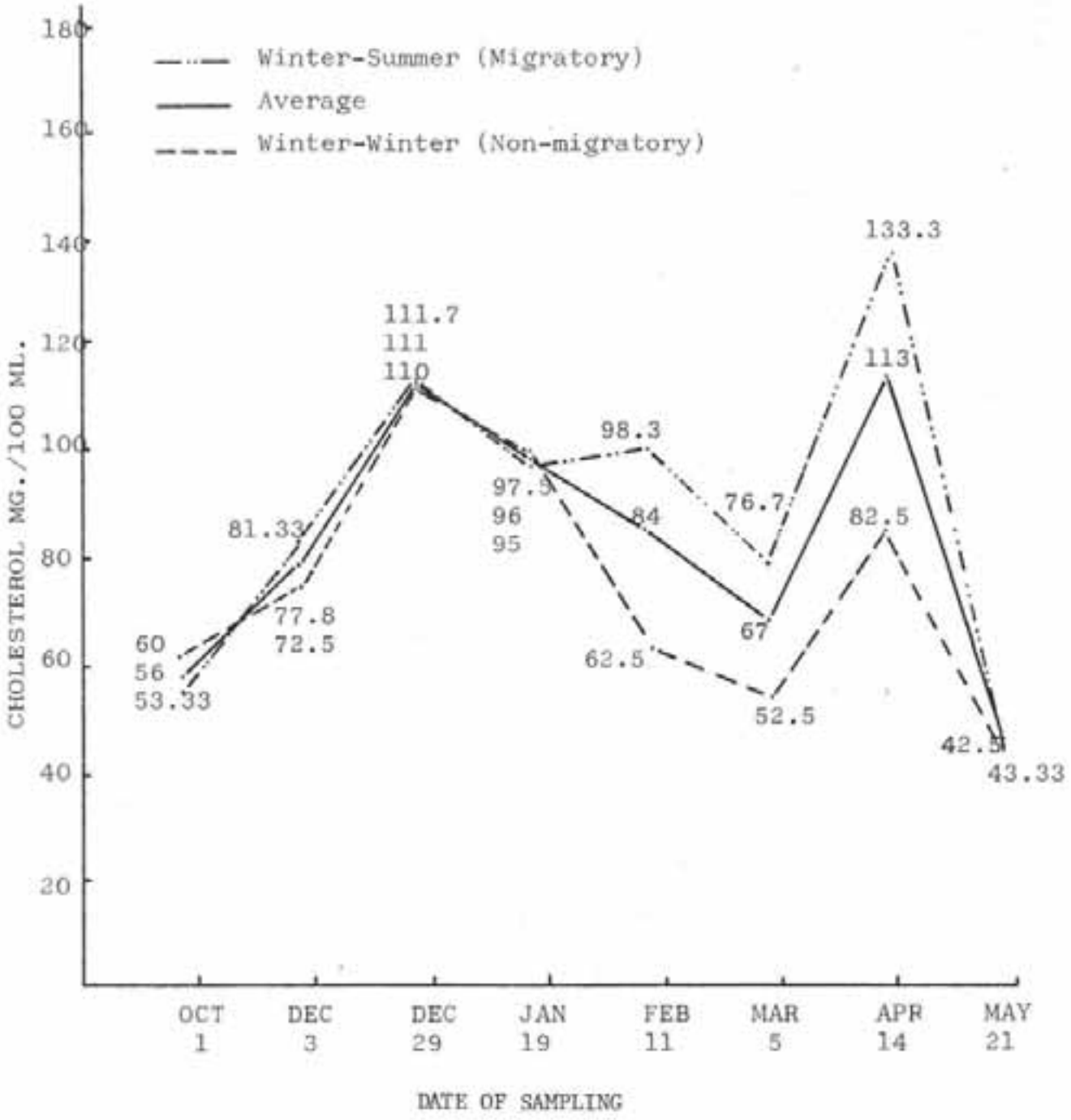

FIGURE 4. Serum chclesterol level changes in bighorn sheep on different quality diets.

BUN - NUTRITION

Seasonal blood urea nitrogen values were related to nutritional measurements shown in Table 1 & 2 for both groups of sheep. Generally, the number of significant relationships increased for both groups as anabolic points were removed and submaintenance diets reflected catabolic processes. Similarly, nitrogen retained/gm of ingested nitrogen and urinary protein/gm of ingested protein are specific indiators of metabolic nitrogen, significant for both groups at $p = .05$ in 4 and 5 computer runs, respectively. More general nutritional measurements were significant in only 1 or 2 computer runs. In addition, more nutritional variables (63 as compared to 41) were significant (digestible protein, body weight, nitrogen retained) for the non-migratory group due to its extensive and more consistent period of catabolism. Although the significance of the change in the number of significant nutritional variables for each computer run is difficult to interpret without additional experimentation, it does appear to be related to the effects of the change-over between anabolism and catabolism. Removal of the effects of the high quality diet (computer run 2) for the non-migratory group increases the number of significant regressions from 3 to 15. However, removal of the first and last (high quality diet) points reduces the number of of significant regression from 15 to 4 due to the effects of the anabolic point (5th point) produced by the multiple vitamin tonic. Removal of the anabolic points (5th and last) increases the significant relationship from 4 to 18. A slight further improvement is provided by removal of the first point which may be partially influenced by the change from anabolism to catabolism. The migratory group, by comparison

SIGNIFICANT BLOOD UREA NITROGEN VALUES FOR THE NON-MIGRATORY GROUP

NUTRITIONAL PARAMETERS	COMPUTER RUN 1		2		3		4		5		
	sig	r	sig	r	sig	r	sig	r	sig	r	Total
Feed intake gm/day							.05	.81			1
Feed intake/Kg BW									.008	.97	1
Feed intake/Kg BW .75									.01	.95	1
Per cent apparent digestibility of dry matter			.04	.79			.003	.95	.01	.96	3
Per cent crude protein in forage			.03	.81			.002	.97	.01	.96	3
Protein intake gm/day							.02	.89	.01	.96	2
Protein intake/Kg BW							.01	.90	.01	.96	3
Per cent digestible protein			.05	.75			.01	.91	.01	.96	3
Digestible protein gm/day			.05	.76			.009	.92	.01	.96	3
Digestible protein/Kg BW			.03	.79			.004	.95	.006	.97	3
Nitrogen balance gm/day			.04	.77			.006	.94	.009	.96	3
Nitrogen retained/gm of ingested nitrogen			.01	.85	.04	.84	.001	.97	.007	.97	4
Nitrogen retained/Kg BW			.03	.80			.004	.95	.009	.96	3
Nitrogen retained/Kg BW .75			.03	.79			.004	.95	.009	.96	3
Per cent crude protein in feces									.02	.94	1
Fecal crude protein gm/day									.02	.94	1
Fecal protein/gm of ingested protein			.01	.85	.04	.84	.001	.97	.007	.97	4
Fecal protein/Kg BW			.04	.77			.01	.91	.01	.95	3
Fecal protein/gm of food intake					.03	.86			.006	.97	2
Urinary protein gm/day									.01	.96	1
Urinary protein/gm of ingested protein	.02	.78	.04	.79	.05	.81	.008	.93	.01	.96	5
Body weight Kg	.03	.76	.05	.76			.007	.93	.03	.92	4
Body weight change Kg											0
Body weight .75	.02	.77	.04	.77			.006	.94	.009	.96	4
Digestible protein gm/day/ Digestible energy meg. cal.			.03	.79			.003	.96	.01	.96	3
Total	3		15		4		18		23		63

TABLE 1. A comparison of the number of significant relationships between nutritional measurements and BUN for the non-migratory group.

NUTRITIONAL PARAMETEPS	SIGNIFICANT BLOOD UREA NITROGEN VALUES FOR THE MIGRATORY GROUP COMPUTER RUN										
	1		2		3		4		5		
	sig	r	sig	r	sig	r	sig	r	sig	r	Total
Feed intake gm/day									.05	.88	1
Feed intake/Kg BW	.05	.70							.05	.87	2
Feed intake/Kg BW .75									.04	.89	1
Per cent apparent digestibility of dry matter	.006	.86									1
Per cent crude protein in forage	.003	.89							.05	.87	2
Protein intake gm/day	.004	.88							.05	.87	2
Protein intake/Kg BW	.003	.89							.05	.87	2
Per cent digestible protein	.02	.79							.05	.88	2
Digestible protein gm/day	.003	.90									1
Digestible protein/Kg BW	.002	.90									1
Nitrogen balance gm/day											0
Nitrogen retained/gm of ingested nitrogen			.04	.79	.03	.85	.03	.84	.03	.91	4
Nitrogen retained/Kg BW									.05	.88	1
Nitrogen retained/Kg BW .75									.05	.88	1
Per cent crude protein in feces	.002	.91							.05	.87	2
Fecal crude protein gm/day	.02	.78									1
Fecal protein/gm of ingested protein					.02	.87			.02	.94	2
Fecal protein/Kg BW	.01	.82									1
Fecal protein/gm of food intake	.03	.75							.05	.87	2
Urinary protein gm/day	.005	.87							.05	.87	2
Urinary protein/gm of ingested protein	.05	.71	.008	.89	.01	.86	.01	.91	.02	.93	5
Body weight Kg									.05	.87	1
Body weight change Kg	.09	.84									1
Body weight .75									.05	.87	1
Digestible protein gm/day/ Digestible energy meg. cal.	.005	.87							.05	.87	2
Total	16		2		3		2		18		41

TABLE 2. A comparison of the number of significant relationships between nutritional measurements and BUN for the migratory group.

experienced changes from anabolism to catabolism within the total sampling period (points 2-5) rather than at either end, as shown by the non-migratory group. Thus, BUN was significantly related to the nutritional variables when all anabolic points were included (computer run 1) or when they were excluded (computer run 5). Exclusion of a single or 2 of 3 anabolic points (computer run 2, 3 and 4) did not improve the level of significance. Sampling for BUN during a metabolic changeover period may produce results which do not adequately describe the condition of the animal or the population.

Preliminary data (Hebert 1973) indicates that fecal nitrogen (per cent crude protein in the feces) may be useful as an indicator of animal condition or population productivity. The potential of this nutritional parameter as an indicator of the status of the animal or population is suggested by its significant relationship with BUN in computer run 1 and 5 ($p = .002$, $r = .91$; $p = .05$, $r = .87$, respectively) for the migratory group and computer run 5 for the non-migratory group ($p = .02$, $r = .94$). These particular computer runs best describe the nutritional status of the group as evidenced by the number of significant relationships (16 to 23).

Interestingly, significant relationships were obtained with BW and $BW^{.75}$ for the non-migratory group (4 of 5 computer runs) which Le Resche et al. (1974) suggest are collective manifestations of nutritional intake. Both BW and $BW^{.75}$ were significant for the migratory group in computer run 5, which is the best nutritional description of this group. Similarly, BUN appears to describe both the protein and energy status of the animal through a significant relationship with the digestible protein to energy ratio. Thus, the best nutritional assessments of each group

(computer run 1 and 5 for the migratory group; r = .87 and computer runs 2, 4 and 5 for the non-migratory group; r = .79, .96, .96, respectively) included this significant relationship.

Previously, Preston et al. (1965) and Somers (1961) demonstrated that protein intake was significantly related to BUN (expressed as CP gm/ $w^{.75}$, r = .99 or as gm/day, r = .97, respectively). This relationship was supported by the work of Lewis (1957) and McIntyre (1970). These studies utilized domestic animals and expressed the relationship at above maintenance levels of CP intake. Similarly, results from this study indicate a significant relationship between BUN and per cent CP in the forage, protein intake in gm/day and protein intake/Kg BW for the migratory group in computer run 1 and 5 (r = .89) and for the non-migratory group in computer run 4 and 5 (r = .90 and .96, respectively).

HEMATOCRIT, HEMOGLOBIN, CHOLESTEROL - NUTRITION

Hematocrit and hemoglobin do not show seasonal trends (Figure 3) and are not significantly related at P = .05, to the measured nutritional parameters for either group or any computer run. Cholesterol was regressed with 9 energy nutritional measurements (gross energy and digestible energy (DE)/day, /Kg BW and /Kg $BW^{.75}$; %DE, DE/gm of feed intake and DP gm/day/DE meg. cal.) in an attempt to assess energy metabolism of the wintering ungulate. There were no significant relationships at the level of P = .05.

POPULATION CONDITION

The use of blood chemistry will allow an assessment of nutritional condition and/or population productivity through seasonal comparison of relative values. However, frequency of sampling necessary to quantify seasonal periods an animal remains at each level of condition, is usually

inadequate to pinpoint specific periods of change in animal growth or metabolism (Figure 5). Thus, it is more important to determine the length of time an animal or population sustains itself at submaintenance levels, if assessment of population productivity is the desired goal than it is to simply determine that they are at submaintenance levels. Consequently, the assessment of condition in Figure 5 may be expressed as PERIOD AT MAINTENANCE OR SUBMAINTENANCE X CONDITION LEVEL (BUN). Similarly, the assessment of productivity of the population may be expressed as: PERIOD OF ACTIVE GROWTH (GREENUP TO BIRTH) X LEVEL OF GROWTH OR CONDITION (BUN) (FECAL N). Population productivity may also be expressed as a response to: PERIOD AT MAINTENANCE OR SUBMAINTENANCE X CONDITION LEVEL (BUN) + PERIOD OF ACTIVE GROWTH X LEVEL OF GROWTH OR CONDITION (BUN) (FECAL N), or as a condition indice of the growth period minus the condition index of the maintenance period.

DISCUSSION

Blood urea nitrogen has been used as a valuable tool in the assessment of above maintenance protein status of the domestic ungulate for several years (Preston et al. 1965, Somers 1961). Only recently has it been examined (Franzmann 1972, de Calesta et al. 1977, Seal et al. 1978a, b) as an indicator of nutritional status of the wild ungulate. To date, BUN has not been adequately related to the below maintenance protein status of a wintering ungulate. Consequently, the BUN curve which is a function of CP intake (Preston et al. 1965, Franzmann et al. 1972) at above protein maintenance levels may have similar BUN values to the BUN curve at below protein maintenance levels. This was not addressed by Le Resche et al. (1974), Seal et al. (1978), or Franzmann (1972) and depending upon the frequency of sampling within the population may produce similar individual values from an increasing or decreas-

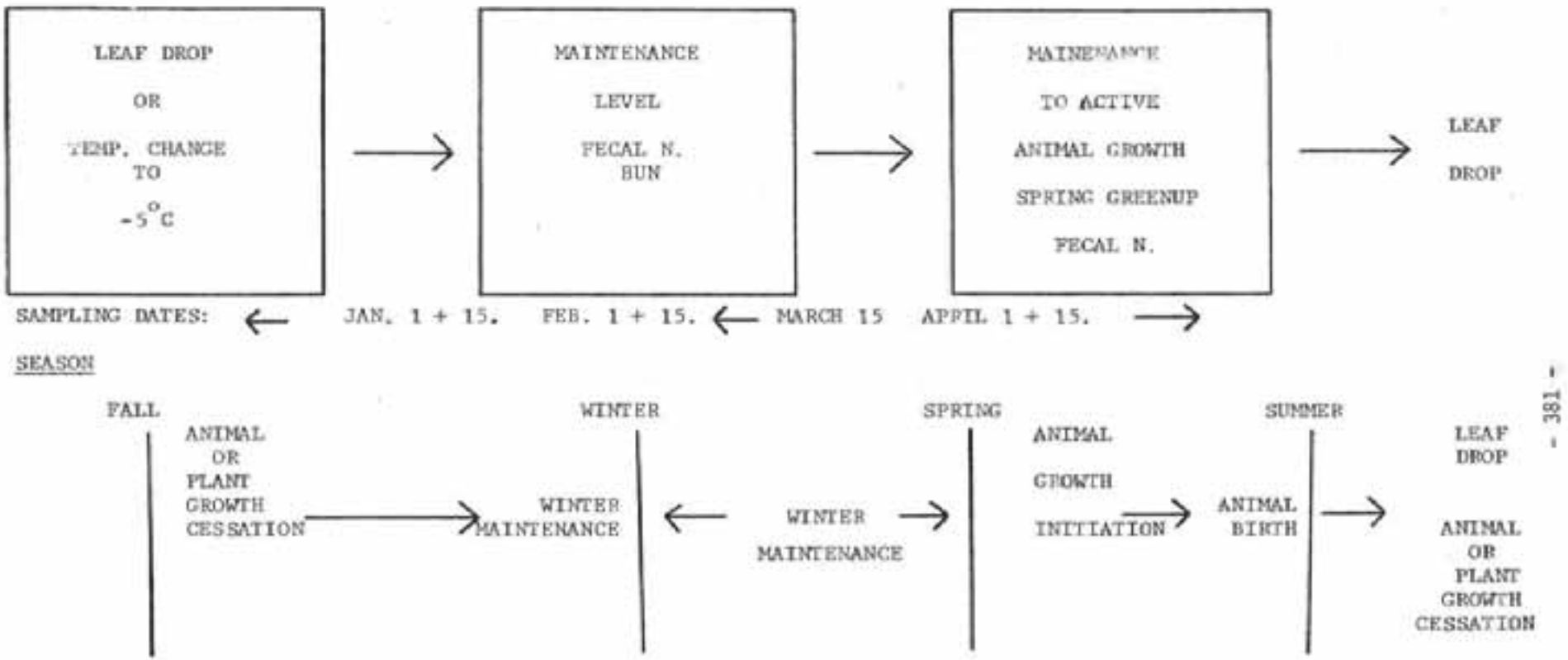

FIGURE 5. The relationship between phenology, animal condition and the seasonal time available for each process.

ing curve, since wild animals lack synchrony of declining physical condition (de Calesta 1977). It will be necessary to accurately determine the physical boundaries and parameters of the population to be sampled.

For the majority of variables, blood chemistry is still at the stage of determining base line values in the face of many uncontrolled field variables. In most studies there are too few samples to standardize for age, sex, season, habitat, climate, and declining forage quality. In some instances (BUN, inorganic phosphorus, calcium, etc.) certain values have been related to crude indices of condition but few studies of wild animals have related blood chemistry to specific nutritional measurements (de Calesta 1977). Currently, the majority of studies have concentrated on comparing relative BUN (Franzmann 1972) or inorganic phosphorus (Hebert 1972) values between seasons in their assessment of change in nutritional status. If blood chemistry is to adequately assess nutritional condition or address the variables of population productivity, recruitment or overwinter mortality, they must quantify the time period in addition to the relative seasonal assessment. Thus, the sequence and intensity of sampling become important criteria in the assessment of nutritional condition. Similarly, complementary sampling programs are important in discerning the anabolic from the catabolic BUN curve. For example, fecal nitrogen can be utilized to indicate whether an animal is above or below the protein maintenance level. In addition, fecal nitrogen is a sensitive indicator of the change between submaintenance levels of winter forage and high quality spring forage. BUN alone or with fecal nitrogen can be used to assess the relative differences between populations in the timing of dietary quality change or the relative magnitude of the change. It is apparent from this study that frequency of sampling

must recognize the timing of changes in dietary quality, the period at maintenance or submaintenance and the period of previous nutrition as it influences the change between anabolism and catabolism. The non-migratory group began to change from a state of anabolism to catabolism around October 1, approximately 3 -3.5 months ahead of the migratory group. Although both groups received the same low quality diet during this period, the previous superior nutrition of the migratory group maintained their nutritional status. Similarly, hemoglobin and hematocrit can be used to indicate nutritional differences between populations or habitat types (Franzmann 1972) but may not be as useful in the assessment of relative magnitude of difference or the assessment of the time period at any particular level of condition. A detailed assessment of nutritional condition as it relates to productivity or winter mortality will undoubtedly require a combination of methods to achieve a satisfactory assessment.

The study of fat metabolism (energy) in relation to blood constituents has received little study (Le Resche et al. 1974). Coblentz (1975) feels that the chemical component used in management should be correlated to the quality of the animals diet and be involved with energy production or storage. de Calesta et al. (1977) found that free fatty acids changed during starvation, indicating that fat stores were mobilized to offset inadequate energy intake.

Coblentz (1975) and Le Resche et al.(1974) suggest that of the lipids, cholesterol may be the most important indicator of animal condition while Le Resche et al. (1974) indicates that both cholesterol and triglycerides are low in Cervids and that triglycerides will probably not be useful. In contrast, Allen (1970) indicates that triglycerides

comprise 83% of the dietary lipids which enter the lacteals while cholesterol, esters and free cholesterol comprise only 7%. However, this study indicated non-significant relationships between cholesterol and specific energy intake measurements, although the seasonal curve and actual values compare favourably with Le Resche et al. (1974) but diverge from Coblentz (1975).

Recently, Seal et al. (1978b) showed a highly significant increase in non-esterified fatty acid levels (NEFA) on low energy diets and suggests that they can serve as an indicator of energy intake in deer. Similarly, Seal and Hoskinson (1978) found a 2-fold difference in serum triglycerides between 2 pronghorn antelope populations differing in nutritional status.

It appears that the energy status of wild ungulate populations should concentrate on NEFA and triglyceride levels and abandon cholesterol values.

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