

series of analyses the weight of carbon dioxide found was 0.21 per cent lower than that computed from the weight of rubber burned, the weight of the water being only 0.06 per cent low. For the four samples of recrystallized rubber, the carbon dioxide was 0.09 per cent low and the water 0.08 per cent high. These differences are in the direction we would expect if the discrepancy in the total weight were the result of probable impurities in the rubber, particularly water and ether; on the other hand, the presence of oxygen combined as hydroxyl would not affect the ratio of hydrogen to carbon, and in the form of other probable radicals it would reduce that ratio.

If the small difference between the theoretical value and the sum of hydrogen and carbon is to be considered significant, the difference between their theoretical and observed ratios must also be significant, and this definitely indicates incomplete purification rather than combined oxygen, as postulated by Midgley.

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## Variation Among Lamb Carcasses in the B Vitamin Content of Meat<sup>1</sup>

SYLVIA COVER, ESTHER M. DILSAVER, and RENE M. HAYS

*Agricultural and Mechanical College of Texas,  
College Station*

It is generally accepted that ruminants do not require the B vitamins. Hence, the variation in ration content may be assumed to have no effect on the B vitamin content of their meat.

Nevertheless, considerable difference in B vitamin content of the raw meat of different carcasses of beef has been reported (1). This difference has been questioned on the grounds that there was no way to remove the variation associated with carcasses from that associated with performing the analyses at different times. In more recent experiments with groups of lamb carcasses, however, there have been findings similar to those in the previous study. The additional evidence, moreover, was obtained from an experimental design improved to meet the previously mentioned criticism.

The experiment was originally planned to overcome as nearly as possible the suspected variation between carcasses. But the variation was notable even under these conditions. Because the details of the entire experiment of which this study is a part will be given in subsequent papers, only those details concerned with carcass differences will be given here.

The samples of meat were obtained as follows: Cubes of meat from a wholesale cut (triangle) were mixed for each of four carcasses separately. Enough cubes to weigh exactly 125 grams were taken from each of the four carcasses and combined to make one 500-gram sample. Equal numbers of 500-gram packages were placed in each of two flat pans and frozen in the deep freezer,

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where they were kept until needed. To obtain replications, this entire procedure was repeated with meat from other lots. Six replications were employed, making a total of 24 lamb carcasses. Storage periods between killing and freezing were held constant, as were those between freezing and analysis, so that these storage periods would not be a possible cause of variation in vitamin content. The same sample was used for the determination of the following B vitamins: thiamine, pantothenic acid, niacin, and riboflavin.

In preparation for testing, one of the two pans in a replicate was removed to the laboratory, where the samples were allowed to thaw in the refrigerator. Two of the samples were analyzed raw, the other samples after cooking. One week later the other

TABLE 1  
VARIATION IN VITAMIN CONTENT BETWEEN REPLICATES  
(GROUPS OF CARCASSES)

Replicate No.	No. of samples	Thiamine	Panto- thenic acid	Niacin	Ribo- flavin
		Actual content (μg./gram dry free-fat basis)			
1	4	8.22	24.75	262.9	12.91
2	4	9.33	25.49	257.4	11.58
3	4	11.72	25.16	231.9	11.37
4	4	7.01	28.65	269.8	10.57
5	4	8.34	24.30	287.2	10.40
6	4	8.15	26.73	270.5	11.57
Greatest difference (highest —lowest).....		4.71	4.35	55.3	2.51
Percentage difference*.....		67	18	24	24
F value of replicates†.....		28.29‡	13.80‡	25.13‡	37.78‡

\* Calculation =  $\frac{\text{greatest difference} \times 100}{\text{lowest replicate}}$

† Calculated from dry fat-free basis.

‡ Error terms were remainder after replicates, order, and O × R were removed.

F values needed for significance were: 0.05 level = 3.11, 0.01 level = 5.06.

pan of samples from the same replicate was removed and the raw samples treated in like manner. Thus, two combinations of the data on raw meat were possible: those associated with order of analysis and those associated with replications. Since the data were collected in this fashion, the variation associated with doing the analyses at different times could be removed from replications by an analysis of variance.

The average vitamin content of each of the 6 replicates is given in Table 1. The variation is shown by the differences between the highest and lowest value, the percentage difference, and the F value. As will be seen, the difference between replicates is highly significant for all of the four vitamins in lamb. The percentage difference in each case is above any recognized value for experimental error. It would appear that the highly significant difference between replicates is associated with something other than the different occasions on which the analyses were made. Carcasses (or groups of carcasses) are apparently the dominant factor associated with replicates. It seems probable that the difference would have been even greater had the analyses been made on individual rather than on groups of carcasses.

That the meat of lamb varies in B vitamin content from carcass to carcass is of unusual interest because lambs are known not to require the B vitamins. Sheep have been shown to synthesize thiamine, pantothenic acid, and riboflavin in the rumen (2), and an excretion study indicates that they also synthesize niacin (3).

There are no clues as to why some of the animals had higher B content in the meat than others, because production histories could not be obtained during the emergency period when this meat was purchased. Whether the factors which control the B vitamin deposition in the meat are environmental or genetic must be determined by further research.

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## The Effects of Cytochrome C in Anoxia

I. HERBERT SCHEINBERG and H. O. MICHEL

*Biochemistry Section, Medical Division,  
Chemical Corps, Edgewood Arsenal, Maryland*

Proger and his co-workers (4) have reported that intravenously administered cytochrome C is capable of mitigating the physiological effects of anoxia. In view of these reports it was thought that cytochrome C might be of value in preventing cyanide intoxication. Experiments to be reported elsewhere failed to demonstrate any significant effect of cytochrome C in cyanide-poisoned rats. Further work was then carried out in an attempt to confirm two findings of Proger and his group which were susceptible to objective and quantitative measurement: the effects of cytochrome C on the organ content of easily hydrolyzable phosphorus compounds, and the survival times of anoxic rats.

The resynthesis of adenosine tri- and diphosphate from adenylic acid and inorganic phosphate is coupled to oxidative processes (3) and should be diminished under conditions of anoxia. The tissue content of adenosine tri- and diphosphate parallels the content of easily hydrolyzable phosphorus (2, 3). In addition, under conditions of anoxia the blood level of lactic acid increases (1). In our experiments the amount of easily hydrolyzable phosphorus in heart and kidney, the blood level of lactic acid, and the measurement of survival times were used to quantitate the effect of anoxia on rats and to measure the value, if any, which cytochrome had in the treatment of anoxia.

In the first experiment both members of pairs of littermate white rats were given 2.0 cc./kg. of physiological saline intravenously. One member was then placed in an atmosphere of 3.9 per cent oxygen, and the other allowed to breathe air. After 8 minutes determinations of the easily hydrolyzable phosphorus ("7-minute phosphorus") of kidney and heart and of the blood lactic acid were made. These revealed a significant difference in all three quantities between those rats which breathed 3.9 per cent oxygen and those which breathed air (Experiment 1, Table 1).

In the second experiment both members of pairs of littermate white rats were placed in an atmosphere of 3.9 per cent oxygen after one had been given approximately 20 mg./kg. of cytochrome C in saline intravenously and the other an equal volume of physiological saline. Determinations of the same

TABLE 1

EFFECT OF CYTOCHROME C ON SEVERAL MANIFESTATIONS OF ANOXIA IN RATS

Experiment	No. of pairs	Treatment of animals	Mean of differences between test and control*	P*
1	7	Pairs of rats (test and control) pretreated with saline. Test placed in an atmosphere of 3.9 per cent oxygen for 8 minutes; control remained in air.		
		Kidney 7-minute phosphorus (mg./100 grams fresh tissue)	-2.37	0.025
		Heart 7-minute phosphorus (mg./100 grams fresh tissue)	-5.07	0.025
		Blood lactic acid (mg./100 cc. blood)	110.8	Less than 0.01
2	11	Pairs of rats (test and control) placed in 3.9 per cent oxygen for 8 minutes. Test pretreated with cytochrome C; control pretreated with saline.		
		Kidney 7-minute phosphorus (mg./100 grams fresh tissue)	0.27	0.68
		Heart 7-minute phosphorus (mg./100 grams fresh tissue)	-0.78	0.41
		Blood lactic acid (mg./100 cc. blood)	-0.64	More than 0.90
3	25	Pairs of rats (test and control) placed in 2.8 per cent oxygen. Test pretreated with cytochrome C; control pretreated with saline.		
		Survival time (min.)	0.66	0.45

\* The differences between the test and control animals with respect to the measured quantities were found, and from these the values of P, representing the probabilities that the observed differences were due to chance, were determined.

three quantities as in Experiment 1 revealed no significant difference between the cytochrome C and saline pretreated animals (Experiment 2, Table 1).

In the third experiment both members of pairs of white rats of the same age, weight, and sex were placed in an atmosphere of 2.8 per cent oxygen after one had been given 5 mg. of cytochrome C in saline and the other an equal volume of physiological saline intravenously. There was no significant difference