they then decline upon these diets. Milk food speedily brings restoration of growth; and it has been shown that the "essential" accessory factor responsible for this effect is a component of the cream which is present in butter.[1] Further experiments now indicate that the butter-fat separated by centrifugal methods from unsalted butter contains the substance which averts the cessation of growth and possible nutritive decline noted when lard is used instead of milk-fat.

Butter-fat thus prepared is free from nitrogen, phosphorus and ash-yielding constituents. The growth-promoting substance therefore is not a phosphatide (lecithin) or an inorganic compound.

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The presence of creatinine in muscle.

(PRELIMINARY PAPER.)

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The presence of creatinine in muscle has, in general, been denied by those who have undertaken a study of this question. In earlier communications[2] upon various phases of the creatine-creatinine problem, evidence has been presented which is strongly in harmony with the metabolic relationship of these two substances. Since creatinine is so rapidly eliminated from the body, its presence in the muscle tissue would not be expected in large quantity. On the other hand, if creatinine originates from creatine, this transformation might be expected to take place in the muscle tissue, and on this account it would seem that, with sufficiently delicate and reliable methods, it ought to be possible

[1] Osborne and Mendel, "The Relation of Growth to the Chemical Constituents of the Diet," *Journ. of Biol. Chem.*, XV, pp. 311–326, 1913; also McCollum and Davis, "The Necessity of Certain Lipins in the Diet During Growth," *Journ. of Biol. Chem.*, XV, pp. 167–175, 1913.

[2] See Myers and Fine, PROC. SOC. EXP. BIOL. AND MED., 1912–13, X, pp. 10, 12 and 168; also *Jour. Biol. Chem.*, 1913, XIV, p. 9; XV, pp. 283 and 305; XVI, p. 169.

to detect the presence of creatinine. We believe we have demonstrated that creatinine does exist in very small quantity in muscle (rabbit), equivalent to about one per cent. of the creatine. The quantity appears to average 6 or 7 mgm. per 100 grams of moist muscle, although variations of 3 to 10 mgm. have been encountered. An observation which is much more significant, however, is that, when the muscle is allowed to autolyze at body temperature under antiseptic conditions, the creatinine content increases at a very uniform rate at the expense of the muscle creatine. One experiment may be cited. The muscle of Rabbit No. 77 had an initial creatinine content of 6 mgm. per 100 grams; at the end of 2 days this had increased to 20.2 mgm., in 4 days to 36.6 mgm., in 6 days to 50.6 mgm., in 8 days to 62.5 mgm., and in 10 days to 74.5 mgm., an average *uniform daily increment* of 7 mgm. per day. This fact, when considered in connection with the uniform content of muscle creatine previously observed by us,[1] is, we believe, the fundamental factor in bringing about the constant daily excretion of creatinine originally observed by Folin. When pure creatine is added to autolyzing muscle it experiences the same fate as the creatine originally present, while in one experiment where creatinine was added in an amount equivalent to the creatine present, it was found to inhibit the usual transformation. This would seem to indicate that the reaction between these two substances is reversible.

The method employed for the determination of creatinine in muscle is briefly as follows: The finely ground muscle tissue is thoroughly extracted with cold water, and to the extract sufficient alumina cream is added to precipitate the proteins (and enzymes). The mixture is then made up to volume, filtered, and a portion of the perfectly clear filtrate is evaporated with the aid of an electric fan to a sufficiently small volume to make the usual colorimetric estimation.

The present preliminary experiments are the beginning of a series which are planned upon this problem.

It is suggested that the above method for creatinine may be of service commercially in ascertaining the age of meat.

In connection with the present work we have observed that

[1] *Loc. cit.*

certain dietary factors (referable to the nature of protein) exert an influence upon the content of muscle creatine. This observation is being further investigated.

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On re-injection with B. tuberculosis or its products and with sera.

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We have stated in several papers read before this Society that extreme vaso-depression was caused by the intravenous injection of split products of bacterial and other origin. These statements were part of an endeavor to elucidate experimentally the mechanism by which foreign organisms and substances may possibly cause intoxication, infection and anaphylaxis by neutralizing pressor secretions, by removing or using up the nourishment of the host, or by destroying the processes upon which depend the host's specificity, vaso-energy and power of reforming foreign bodies into substances like its own constituents, which may be essential in these conditions to its existence.

This view includes the action of those foreign substances and ferments upon the host, by which the host organism or substrata gives off split products which thus produce an auto-intoxication.

These notes carry this view still further by means of a few observations on death and symptoms following re-injection in animals which have already been injected with tubercle bacilli or products of the tubercle bacilli and with sera. An explanation is advanced that the death which followed the re-injection of the minute dose of *B. tuberculosis*, with the long interval between the first and second injection, namely twelve months, is probably due to a deferred anaphylaxis or persistent increased susceptibility or sensitiveness which may be present for an unusually long time in some of the cases which have apparently recovered from the first injection. There were not enough tuberculous lesions found in this case to satisfactorily account for death.