scribed by Cullen and Wilkins,[1] on duplicate ashings which afforded sufficient material for duplicate determinations of most of the substances in each ash.

Of all the tissues, the uterus was uniformly the richest in sodium and poorest in potassium. The total phosphorus content of both uterus and bladder was decidedly lower than that of skeletal and heart muscle. The findings for skeletal and heart muscle, including certain small constant differences in the composition of the right and left ventricles, were in general agreement with those reported by Cullen, Wilkins, and Harrison[2] and Wilkins and Cullen[3] for human tissues. These authors made no analyses of human bladder and uterus. It is recognized that the presence of blood introduced a slight error but this probably does not invalidate the result.

While the concentrations of the individual bases in the 5 tissues show much variation the sums of the bases in milli-equivalents vary within a comparatively narrow range.

7463 C

Effect of Hydrogenated Fat on Abnormal Carbohydrate Respiratory Quotients of Rats on a Fat-Deficient Diet.

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One of us reported[1] that extremely small amounts of fats have an influence on metabolism greatly in excess of their effect as fat *per se* because of a possible new dietary factor present in the fats. Evans and Burr[2] announced the discovery of a syndrome characterized by subnormal growth and retardation or suppression of ovulation, which developed in rats maintained on a fat-deficient diet. Burr and Burr[3] described additional symptoms of this fat deficiency disease in rats as scaly skin, necrotic tail, and hemorrhagic kidneys, and recognized[4] the fat-contained dietary factor as linoleic acid. A

1 Cullen and Wilkins, *J. Biol. Chem.*, 1933, **102**, 403.

2 Cullen, Wilkins, and Harrison, *J. Biol. Chem.*, 1933, **102**, 415.

3 Wilkins and Cullen, *J. Clin. Invest.*, 1933, **12**, 1063.

1 Wesson, L. G., *Am. J. Physiol.*, 1927, **81**, 513; *J. Biol. Chem.*, 1927, **73**, 507.

2 Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 390.

3 Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

4 Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

cooperative investigation by one of us (W) with Dr. Burr indicated that while linoleic acid is curative for the abnormalities described by Evans and Burr and Burr, it is not curative for the metabolic abnormality described by Wesson.[5]

In the present paper, a comparison is made of the effects of an active natural fat containing linoleic acid on this abnormal metabolism before and after hydrogenation. If hydrogenation does not modify the activity of this fat, a further indication is thereby obtained that linoleic acid is not the active constituent of the fat with respect to carbohydrate metabolism.

The symptom of a deranged metabolism that appears in rats maintained on a diet deficient in fat consists of an abnormal tendency to form fat from carbohydrate, which may be alleviated by small amounts of an active fat.[6] This abnormal tendency to form fat from carbohydrate is detected by means of respiratory quotient determinations obtained during the 6 hours following the feeding of a definite amount of carbohydrate. The normal tendency to form fat from carbohydrate due to over-filling of the glycogen stores is avoided by a preliminary 18-20 hour fast. Reference is made to preceding papers[6, 7] for details of the fat-deficient diet, procedure, and previous findings.

The alcohol-soluble fraction of lard[6] was the fat used in the present work. The iodine number (Wijs) of this fat was lowered by hydrogenation[8] from 80 to 31, and a sample after saponification gave only traces of a petroleum ether-insoluble bromide (0.03%) indicating that most of the linoleic acid had become at least partially hydrogenated. The fat before hydrogenation was liquid at room temperature; after hydrogenation it required a temperature of 50-55°C. to soften and melt.

In order to avoid the effect of small amounts of possibly unchanged active substances in the hydrogenated fat, it was fed, in addition to the fat-deficient diet, to a group of 9 abnormal rats at a level that had been found to be a moderate one for this fat before hydrogenation, that is, 0.14 gm. per 100 gm. body weight daily for 2 weeks.[6] Another group of 7 abnormal rats received the same amount of the original alcohol-soluble fraction of lard, and still

[5] Wesson, L. G., and Burr, G. O., *J. Biol. Chem.*, 1931, **91**, 525.

[6] Wesson, L. G., and Murrell, F. C., *J. Biol. Chem*, 1933, **100**, cii; 1933, **102**, 303.

[7] Wesson, L. G., *J. Biol. Chem.*, 1933, **100**, 365.

[8] Skita, A., and Meyer, W. A., *Ber. chem. Ges.*, 1912, **45**, 3579; Voorhees, V., and Adams, R., *J. Am. Chem. Soc.*, 1922, **44**, 1397.

another group of 6 abnormal rats was given the same amount of the fat after it had been aerated at 100°C.[9]

The abnormal rats, before the addition of fat to their diet, gave R. Q. curves the average area of which above the line R. Q. = 1.00 was 1.31 sq. in. (1 in. = 1 hour and 0.1 unit R. Q.). After the addition of unhydrogenated fat to the diet, a group of these rats gave R. Q. curves, the average area (A, Fig. 1) of which above the line R. Q. = 1.00 was 0.20 sq. in., while the corresponding area (B, Fig. 1) given by another group of rats that received the hydrogenated fat was 0.12 sq. in. In marked contrast to these are the R. Q. curves given by the 3rd group of abnormal rats after

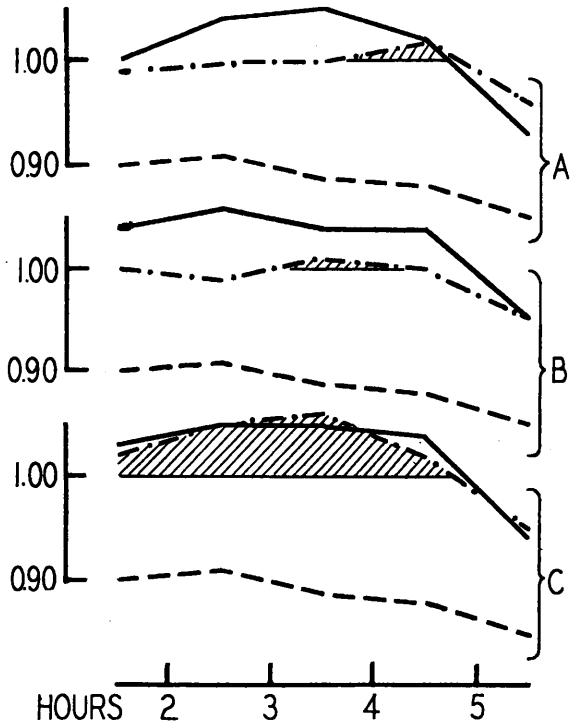

FIG. 1.

R.Q.'s following the feeding of dextrin. Solid lines represent the average R.Q. curves of the abnormal rats before the addition of fat to their diet; dots and dashes, after the addition of small amounts of fat to the diet; and dashes, the corresponding curve given by rats fed a normal diet. The cross-hatching represents the areas of definite fat formation by the abnormal rats after the inclusion of the fats in their diet.

In "A" the fat is the alcohol-soluble fraction of lard; in "B," the same fat after hydrogenation; and in "C," the same fat after aeration at 100°.

[9] McCollum, E. V., Simmonds, N., and Shipley, P. G., *J. Biol. Chem.*, 1922, **53**, 293.

receiving the aerated fat for 2 weeks. The area above the 1.00 line of the average of these curves was 1.33 sq. in. (C, Fig. 1). Thus the aerated fat had little or no effect on the abnormal R. Q. curves, while the unhydrogenated and hydrogenated fat had a marked and similar effect on these curves.

Conclusion. The partial hydrogenation of the alcohol-soluble fraction of lard, leading to the complete or almost complete destruction of the linoleic acid contained therein, does not diminish the effectiveness of the fat with respect to the lowering of the abnormal respiratory quotients of rats on a fat-deficient diet. Further indication is thereby given that the carbohydrate factor postulated as present in natural fats is not linoleic acid, the lack of which in the diet of rats has been found to produce other deficiency symptoms.

7464 P

Penetration of Potassium Into Nitella.

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Experiments with *Valonia macrophysa* indicate that in the light the rate of entrance of potassium is increased when the external concentration of potassium or the external pH is raised above normal, but if they are reduced sufficiently below normal potassium leaves the cells. In the dark the cells fail to grow and potassium does not increase even when the concentration of potassium and the pH are simultaneously increased in the external solution.

These results have been explained by assuming that potassium enters the protoplasm chiefly as KOH, so that the rate of entrance depends on the difference between the ionic activity product [K] [OH] inside and outside.

Similar experiments with *Nitella flexilis* give the following results*: *(a)* The entrance of potassium is relatively rapid in both the dark and the light. *(b)* No growth occurs during the experiments either in the dark or the light, so that the rate of entrance is measured by the increase in the concentration of potassium. *(c)* The rate of increase of potassium is practically independent of the ex-

* The technique resembles that previously described but the analyses were carried out according to the methods of Emich.