The experiment proves the economical use of carbohydrate during periods of work. On the contrary, when 700 grams of meat were given and the dog was compelled to run, the heat production was increased by that quota which would have been added from the specific dynamic action of the protein metabolized. The latter observation confirms Rubner; the first observation has never been reported. The principles are of importance in the proper arrangement of dietaries for those who execute mechanical labor.

58 (1236)

The cerebrosides of brain tissue.

By P. A. LEVENE and C. J. WEST.

[*From the Rockefeller Institute for Medical Research, N. Y.*]

Since the work of Thudichum, evidence has existed that there was more than one cerebroside. On the basis of physical properties and analyses, Thudichum distinguished two cerebrosides, phrenosin and kerasin. He had the correct intuition as to the points of difference of the two cerebrosides, and suggested that this difference was in the nature of one component, namely, the fatty acid. As far as he worked, the other components were identical in all cerebrosides. Unfortunately his method of separation was imperfect. Therefore, each fraction contained a large portion of the other. When the fatty acids were obtained, they analyzed approximately for a stearic acid, which Thudichum named *neuro stearic acid.* Subsequent workers improved the methods of purification particularly that of phrenosin; this permitted the isolation of the fatty acid, *cerebronic acid.* This acid was identified by Thierfelder as an hydroxy pentacosanic acid, $C_{25}H_{50}O_3$. The further work on cerebrosides brought nothing new as regards their structure. The attempt of Thierfelder to classify them on the basis of their sugar contents was futile. Finally, it was possible to isolate from a cerebroside corresponding to Thudichum's kerasin, a fatty acid of different structure from cerebronic acid, namely, lignoceric acid. This acid was isolated nearly simultaneously in three laboratories, but its relation to cerebronic and lignoceric acids was first established in our laboratory, and independently of us by Rosenheim. Since then, it has

been possible to judge of the purity of a cerebroside by the composition of the fatty acid fraction obtained on hydrolysis. This standard has been employed by us in testing the purity of the substance designated as phrenosin and kerasin.

By methods employed in different laboratories, it has been possible to obtain phrenosin in a fair degree of purity. Upon hydrolysis of phrenosin of the highest dextrorotation one obtains cerebronic acid. With the progress of purification the dextrorotation of phrenosin increases. On the other hand, the kerasin fraction acquires an increasing levorotation. Hence one can also be guided in purifying the kerasin by the magnitude of its levorotation. This method has been employed in our laboratory and also by Rosenheim; Rosenheim used in addition to this the selenite plate test. Guided by these tests, Rosenheim obtained substances which showed levorotation of -2 to $-3.5°$, and claimed them to be pure kerasin. On hydrolysis of these fractions he obtained only lignoceric acid.

Several years ago in our laboratory, hydrolyses were made on substances with the same and even higher rotatory power than those employed by Rosenheim. Using methods of hydrolyses different from the conventional method introduced by Thudichum, and used by all other workers, it was found that these fractions contained not only lignoceric acid but also cerebronic acid. From this it was concluded that kerasin had not, as yet, been prepared in a pure state. These results were not published because attempts to isolate kerasin in larger amounts by the methods then used, were not successful, and it was desired to obtain a more satisfactory method for preparation of the cerebroside.

It was thought that by the fractionation, not of the cerebroside mixture itself, but of an acyl derivative, one might obtain more satisfactory results. With this object in view the acyl derivatives of phrenosin and fractions corresponding to kerasin were prepared, as follows:

	M.	$[\alpha]_D^{20}$.	C.	H.
Hexa-acetyl phrenosin	41–43°	−11.08	65.31	9.27
Hexa-acetyl kerasin	54–56°	−16.46	67.00	9.95
Tri-benzoyl phrenosin	65–66°	+21.20	73.45	8.83
Tri-cinnamoyl phrenosin	69–70°	+21.72	73.22	9.45
Tri-*p*-nitrobenzoyl phrenosin	94–96°	+12.18	63.61	7.99

It was found that the benzoyl derivative of phrenosin was more insoluble in methyl alcohol, than that of kerasin. Indeed, by preparing the benzoyl derivative of a mixture with a rotation of ± 0.0 (in pyridine) one could separate this into two fractions, the more soluble of which had a rotation of − 2.25 to 3.2°, varying in different experiments. These rotations refer not to the benzoyl derivatives, but to the cerebrosides obtained after saponification of the acyl derivative. The substance with the highest levorotation (− 3.28°) obtained in this way corresponded to the one named by Rosenheim pure kerasin and the one we had in hand several years ago. Since that time the methods of hydrolysis have been improved; applying this for the analysis of new material, we again found that the fatty acid fraction contained both lignoceric and cerebronic acids. On the basis of this, we confirmed our original view that so-called kerasin has not yet been obtained in the pure state. On the other hand, the very gratifying results obtained by the benzoyl method carries confidence in the possibility of a successful separation of kerasin by repeated benzoylation of the levorotatory fraction of cerebrosides. The work in this direction will be undertaken as soon as a sufficient quantity of the cerebroside mixture will be prepared.

We wish to refer briefly at this place also to the problem of the sugar contents of cerebrosides. On the basis of the present assumption and their structure, the theory requires a value of 20 per cent. Employing Thudichum's method of hydrolysis Rosenheim reports a theoretical yield of sugar. This first seems mysterious to us. Control experiments on pure galactose carried out in our laboratory by G. M. Meyer showed that the sugar subjected to the treatment employed by Thudichum, Rosenheim and others suffered a very considerable destruction. Indeed, in the early part of the work, we could never obtain the required 20 per cent. For a time this seemed to be in the way of accepting the conventional theory of the structure of the cerebrosides. However, conditions of hydrolysis have been perfected in which the sugar undergoes little decomposition, and under such conditions the yield of sugar is as the theory requires, about 20 per cent.