each experiment are recorded in Table 1 along with the initial weights of the rats and the disintegrations per minute in the acetates. The ^{14}C specific activity in each carbon is expressed as the percentage of the sum of the specific activity in both carbons and hence the percentages in the two carbons sum to 100%. Before degrading each acetate, an aliquot was combusted to CO_2 and the specific activity of the CO_2 was determined. Percent recovery is 100 times the specific activity of the ^{14}C in carbon 1 and the ^{14}C in carbon 2 divided by the specific activity of the ^{14}C in the carbons in the aliquot combusted. These recoveries range from 97 to 105%, giving good evidence for the purity of the acetates and the adequacy of the degradations.

In each of the three experiments, clofibrate decreased the quantity of [1-^{14}C]acetate relative to [2-^{14}C]acetate formed from [16-^{14}C]palmitic acid. Therefore, clofibrate decreased the fraction of fatty acid oxidized initially via omega-oxidation. From the distributions in acetates from the control rats one can estimate that 28–56% of the initial metabolism of the fatty acid was via omega-oxidation and that in the clofibrate-fed rats 16–31% was via omega-oxidation [11]. These estimates are for contributions of omega-oxidation in the cellular environment in which the acetylation of phenylaminobutyric acid occurs. The percentage in carbon one varied in the control rats from 21 to 42%. However, in the experiment done in triplicate, the distributions were essentially identical among the three rats. The reason for variations between experiments is uncertain, but we have observed such variations in the past [11, 16]. The variations do not detract from the clear effect of clofibrate on the distributions. The distributions in acetate were not different between the control and ethanol-fed rats. Thus, ethanol administration did not alter the fraction of fatty acid initially oxidized via omega-oxidation relative to beta-oxidation.

In summary, clofibrate, but not ethanol, administration to the rat decreased the proportion of palmitic acid oxidized initially via omega-oxidation as compared to beta-oxidation.

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New method to increase the serotonin level in brain by carotid injection of desoxyfructo-serotonin in mice

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Serotonin (5-HT) has multiple functions in blood and brain [1]. If the level of serotonin can be increased by suitable means, it could be valuable in the treatment of diseases associated with a decrease in serotonin.

Desoxyfructo-serotonin (1-desoxy-1-[5-hydroxytryptamino]-D-fructose or DF-5HT) (Fig. 1) is a sugar derivative of serotonin [2] which seems to cross the blood–brain barrier, since central effects were observed with this compound after intravenous as well as intraventricular administration [3]. It was, therefore, considered necessary to obtain data on the penetration of desoxyfructo-serotonin from the blood to the brain and study its metabolism in this compartment.

Materials and methods

The experiments were performed on male Swiss albino mice weighing between 24 and 26 g. [2-^{14}C]-5-Hydroxytryptamine binoxalate (54 mCi/mmole) obtained from New England Nuclear (Boston, MA) was used for the preparation of desoxyfructo-[2-^{14}C]-5-hydroxytryptamine oxalate [2]. Purity of the radioactive material was controlled by TLC. Solution in 0.9% NaCl of 2.3 mM [2-^{14}C]-DF-5HT, having 0.5 mCi/mmole activity, was freshly prepared and 0.125 ml of the solution was injected into the carotid artery [4]. Following injection, at various intervals from 2 min to 60 min, the radioactivity was assessed in the brain.

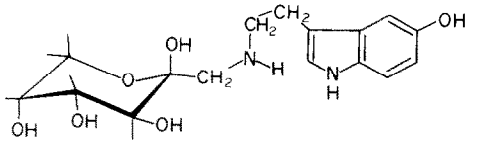

Fig. 1. Desoxyfructo-serotonin.

Whole brain samples were individually homogenized [5] in an Potter–Elvejhem homogenizer in 5 vol. of ice-chilled 10% aqueous solution of trichloroacetic acid (TCA) or in 1% KCl. TCA-extracts were obtained by centrifugation at 10,000 *g* of the above TCA-homogenates of brain, and the supernatants assayed. The radioactivity was counted in an Intertechnique Type 130 Instr.

The effectiveness of both methods was controlled by internal standards. Using the TCA extraction technique, the effectiveness of the method was 28%, using the KCl homogenates it was 70%. The results given in Table 1 were calculated with these yields, and represent the means of the values obtained with both methods.

Determination of total radioactivity in brain. Measurement of total radioactivity was performed on 0.1 ml TCA-extracts in 16 ml Bray's solution [6]. Two minutes after carotid injection the recovery of radioactivity in the brain was 0.6%, after 60 min only 0.33%. The same measurement was repeated with the 1% KCl homogenate, dissolved in an equal volume of CNS tissue solubiliser, Amersham. The recovery of radioactivity was nearly the same (0.77% after 2 min and 0.4% after 60 min), proving the validity of the results obtained with the TCA-extraction procedure.

Separation and identification of [^{14}C]-DF-5HT and its metabolites by high voltage electrophoresis. TCA-extracts of brain were submitted to high voltage electrophoresis [7] in 1% sodium tetraborate buffer (pH 7), on Whatman 3 MM paper, at 5000 V and 300 mA for 15 min, on a Gilson Model-D apparatus. The migration of DF-5HT and its metabolites was followed by coloration by ninhydrin*, using non-radioactive DF-5HT, 5-HT and HIAA as internal reference (Fig. 2).

For counting the amount of the radioactive components, the previously dried paper bands were cut in three 3 cm pieces and placed in 13 ml toluene scintillation fluid. The identity of metabolites was controlled by TLC [8], after elution of the three 3 cm paper pieces by 1:1:1 10% aqueous TCA–ethyl acetate–toluene solvent mixture. The results obtained are shown in Table 1.

The occurrence of 'binding' to protein was controlled by dissolving the corresponding ninhydrin positive spots in 0.2% SLS (sodium lauryl sulfonate). The solution was again submitted to high voltage electrophoresis. The ninhydrin positive material still had not moved with high voltage electrophoresis, showing it to be a high molecular-weight protein.

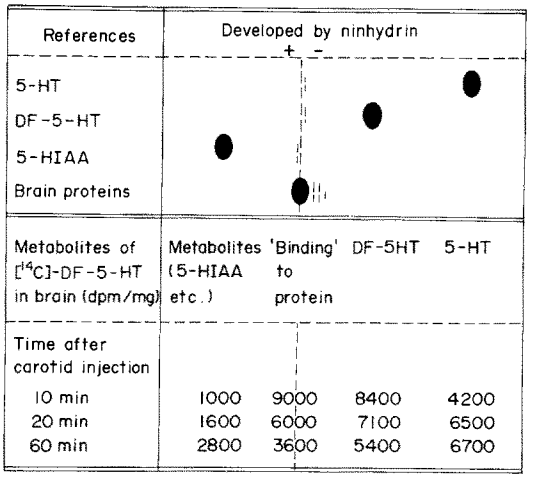

Fig. 2. Separation and identification of [^{14}C]-DF-5HT and its metabolites by high voltage electrophoresis.

Results and discussion

The results relative to the brain level of DF-5HT and its metabolites clearly show, that at 10 min the level of DF-5HT is high in the brain, while the level of 5-HT and other metabolites is low. At 20 min DF-5HT is converted in higher amounts to 5-HT, which increases at 60 min to nearly double that of the normal level [9, 10]. The high level of DF-5HT still observed in the brain at 60 min, may be the result of release of DF-5HT from initial 'binding' to proteins.

Like other Amadori type sugar derivatives [11], desoxyfructo-serotonin has been shown to be a relatively stable compound both *in vitro* and *in vivo* [12]. Therefore, the time taken for the metabolic disposition of the sugar derivative should be long.

The results clearly demonstrate that DF-5HT enters the brain and is slowly converted into serotonin and its metabolites, increasing the available serotonin level.

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* 1% ninhydrin and 3% acetic acid in ethanol, developed by heating for 10 min at 60°.

Table 1. Brain breakdown of [^{14}C]desoxyfructo-serotonin injected in the carotid of mice

Number of animals	Time after injection	Metabolites (5-HIAA, etc.)	Binding*	DF-5HT	5-HT	Total of 5-HO-indoles
3	10 min	0.17 ± 0.02	1.50 ± 0.15	1.40 ± 0.30	0.70 ± 0.08	3.77 ± 0.55
3	20 min	0.25 ± 0.03	1.05 ± 0.06	1.20 ± 0.20	1.08 ± 0.10	3.58 ± 0.29
3	60 min	0.50 ± 0.08	0.60 ± 0.10	0.90 ± 0.10	1.10 ± 0.16	3.10 ± 0.54

Values estimated by high voltage electrophoresis and expressed in μg of the equivalent of 5-HT calculated per 1 g brain with standard deviation.

* Bound to brain proteins.

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Effect of cycloheximide on the incorporation of [^{14}C]glucosamine into UDP-*N*-acetylglucosamine, cell free and protein-bound *N*-acetylneuraminic acid, and plasma membrane glycoproteins of chicken liver

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There are extensive reports dealing with the effect of cycloheximide on various macromolecular biosyntheses. A transient inhibition of protein synthesis in rat liver, followed by a recovery period was established with non-lethal doses of cycloheximide [1–4]. This antibiotic was used for investigating the secretory pathway of rat serum glycoproteins, and the localization of newly formed proteins within the endoplasmic reticulum [5]. The effect of the inhibitor was followed by glycosphingolipid biosynthesis in phytohemagglutinin stimulated human lymphocytes [6].

There is no information whether the glycosylation of nascent polypeptides is coupled with a continuous protein synthesis and whether the blocking in protein synthesis causes some alterations in the content and biosynthesis of nucleotide sugars, the direct carbohydrate donors in the glycoprotein, and glycolipid formation.

The present study was undertaken in an effort to investigate the effect of cycloheximide (CHI) on the incorporation rate of [^{14}C]glucosamine into UDP-*N*-acetylglucosamine, cell free *N*-acetylneuraminic acid (*N*-acetylneuraminic acid + CMP-*N*-acetylneuraminic acid), and protein-bound *N*-acetylneuraminic acid, into plasma membrane glycoproteins from chicken liver.

All studies were performed using 50–70 g white leghorn chickens. Cycloheximide at a dose of 2 mg/kg body wt [7] was administered intraperitoneally 30 min before the labelling *in vivo* (3 μCi/100 g body wt) with [^{14}C]glucosamine (sp. act. 3.4 mCi/mmole; Radiochemical Centre, Amersham, UK) or with [^{14}C]leucine (sp. act. 300 mCi/mmole; Radiochemical Centre) in a dose of 20 μCi/100 g body wt. The animals were killed by decapitation 2 hr after the isotope injection. This time point was chosen because our preliminary experiments with [^{14}C]leucine [8] have demonstrated that at the given dose CHI inhibits the incorporation of the label into chicken liver plasma membrane proteins more than 90%. In the present study the radioactive proteins were prepared for counting by earlier methods [9]. The removal of livers, the isolation of plasma membranes and of radiochemically pure UDP-*N*-acetylglucosamine, were described previously [10]. The separation and purification of cell free and protein-bound *N*-acetylneuraminic acid (NANA) was achieved according to the procedure described elsewhere [11]. For measuring of the radioactivity associated with the acid-insoluble plasma membrane fraction (plasma membrane glycoproteins) the procedure [12] was applied. The membrane preparations were treated with 20% (w/v) trichloracetic acid (5 ml for 3–4 mg membrane protein). After centrifugation, the sediment was washed with 10% trichloracetic acid, once with methanol/ether/chloroform (1:1:2 by vol.), twice with 95% (v/v) ethanol and dried overnight at 37°. Five milligrams of the dried sample was dissolved in 0.2 ml of NCS solubilizer at 50°, then 10 ml of 0.6% 2,5-diphenyloxazol in toluene was added. To the samples of UDP-*N*-acetylglucosamine, cell free and protein-bound NANA, 5 ml of a mixture containing 1 vol. of Triton X-100 and 2 vol. of toluene PPO/dimethyl POPOP phosphor were added. The radioactivity was determined in a Packard Tricarb 3330 scintilation spectrometer.

Analysis of the effect of CHI on the incorporation of