mic shift in acidic medium, suggested that trigilletimine was a benzylisoquinoline-benzyltetrahydroisoquinoline dimeric alkaloid of the dibenzodioxin type containing 2 methoxy and 1 N-methyl groups.

N-methyltetrahydrotrigilletimine (**3**) was shown to be the enantiomer of N-methyltelobine[5] (**6**) (mp 175–180° dec; $[\alpha]_D^{18}$ +248° [$CHCl_3$]) by direct comparison (uv, ir, nmr, ms, mp. $[\alpha]_D$). Since N-methyltetrahydrotrigilletimine (**3**) is the enantiomer of N-methyltelobine (**6**), tetrahydrotrigilletimine must be represented by **2** or **7**. This was further substantiated by the fact that tetrahydrotrigilletimine is not identical to trilobine[4] (**4**), *O*-methylmicranthine[5] (**5**) or telobine[5] (**8**) or their enantiomers by comparison (mp, ir, nmr, $[\alpha]_D$). Since telobine (**8**) is the enantiomer of **7**, and neither are identical to tetrahydrotrigilletimine, this indicates that tetrahydrotrigilletimine must be represented by **2** and therefore trigilletimine by **1**. It is of interest to note the stereospecific reduction of trigilletimine to tetrahydrotigilletimine. A consideration of models reveals that this may be due to a particularly stable conformation which subsequently encourages hydrogenation in a stereospecific manner. To our knowledge, this is the first example of a naturally occurring dibenzodioxin alkaloid containing an aromatized isoquinoline ring (which has only been found in one other *bis*benzylisoquinoline alkaloid, the quaternary base phaeantharine chloride from *Phaeanthus ebracteolatus*[7]).

Summary. The structure of trigilletimine (**1**), a new *bis*benzylisoquinoline dibenzodioxin alkaloid from *Triclisia gilletii* (De Wild.) Staner and *T. patens* Oilv., was determined by spectral means and conversion to the N-methyltetrahydro derivative.

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Changes in Major Liver Constituents Following Hypophysectomy in Goldfish (*Carassius auratus*)[1]

Hypophysectomy in teleosts results in species specific changes in liver glycogen levels. In *Anguilla rostrata* liver glycogen was depleted[2] whereas in *Poecilia latipinna* it was elevated[3]. In the latter species, however, comparisons were made with unoperated and not sham-operated fish, thus leaving unaccounted the stress of operation. *Tilapia mossambica*, on the other hand, showed no change in liver glycogen levels following hypophysectomy[4]. However, hypophysectomy can alter liver size as well as glycogen concentrations as shown in *Fundulus heteroclitis;* glycogen concentrations dropped slightly after pituitary removal, but liver size almost doubled resulting in a net accumulation of glycogen[5]. Again comparisons were made with unoperated rather than sham-operated controls. With this in mind we have examined the quantitative and qualitative changes in major constituents of goldfish (*Carassius auratus*) liver following hypophysectomy.

Materials and methods. Medium size goldfish (4–9 g) were obtained from Hartz Mountain Pet Supplies, Rexdale, Ontario and maintained at 20 ± 0.5 °C for at least 1 month prior to operation. Operated and unoperated fish were held in 20 °C tap water. NaCl was added to the water giving a 0.2% solution to insure against osmoregulatory failure in hypophysectomized fish. The fish were fed Purina trout ration daily except on the day of killing. The methods of hypophysectomy and sham operation were reported earlier[6]. Completeness of hypophysectomy was determined by the colour-loss criterion of JOHANSEN and ROY[7] and the absence of any pituitary remnants under moderate magnification at autopsy.

In the 5th week after operation fish were killed by decapitation and their livers were analyzed immediately for their constituents. Lipid content was estimated by the method of BLIGH and DYER[8]. Glycogen was determined by the method of Lo et al.[9] with slight modification. It was found that the glycogen isolation step effected by precipitation with ice-cold ethanol and centrifugation could be omitted without significant change in yield (Table). Protein was assayed by the method of LOWRY et al.[10] and the biuret method[11]. Addition of biuret reagent to the TCA precipitate of diluted ground liver homogenate resulted in a persistent cloudiness which was

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Summary of liver constituents from normal, sham operated, and hypophysectomized *Carassius auratus*

Parameter	Unoperated	Sham operated	Hypophysectomized
Liver somatic index (mg liver/g of fish × 100)	3.83 ± 0.24 (10)	4.02 ± 0.33 (12)	7.80 ± 0.52[a] (12)
Dry weight	314 ± 4 (10)	321 ± 7 (12)	315 ± 4 (12)
Glycogen (alkaline digest)	198 ± 4 (10)	194 ± 3 (12)	253 ± 3[a] (12)
Glycogen (ethanol precipitate)		192 ± 5 (10)	252 ± 3 (10)
Protein (Biuret)	103.0 ± 3.2 (10)	95.9 ± 3.3 (12)	50.6 ± 2.9[a] (10)
Protein (Lowry)	87.6 ± 3.4[c] (10)	86.0 ± 3.1[b] (10)	46.6 ± 2.4[a] (11)
Lipid	31.3 ± 1.8 (12)	27.9 ± 1.1 (13)	15.4 ± 1.0[a] (14)

Data are expressed as mean ± standard error (sample size), and all values except liver somatic index are mg/g wet wt. of liver. [a] Significantly different from unoperated and sham operated, $p < 0.05$. [b] Significantly different from biuret method in the same group, $p < 0.05$. [c] Significantly different from biuret method in the same group, $p < 0.005$.

eliminated by the addition of 0.2 ml of a 4% sodium deoxycholate solution giving a final volume of 4.2 ml.

One-way analysis of variance was performed to determine the significance of the results. Where significance occurred comparison between groups was made by the Newman-Keuls multiple range test. Significance between the two techniques for each of glycogen and protein analysis was determined with the two-tailed Student's *t*-test.

Results and discussion. Results are shown in the Table. The liver somatic index and glycogen concentration were significantly higher, while the concentrations of lipid and protein were significantly lower in hypophysectomized fish than in unoperated and sham-operated fish. There were no significant differences between unoperated and sham-operated fish.

Since the phenol-sulfuric acid reagent reacts with many sugars[12] the usual procedure for glycogen analysis using an ethanol precipitate measures the quantity of alkali soluble polysaccharide which is presumed to be mainly glycogen[13]. Direct analysis of the alkaline digest is potentially prone to error if interfering substances react with the phenol-sulfuric acid reagent. However, the results comparing the ethanol precipitate with the alkaline digest indicate no significant difference between the two procedures for goldfish liver. The glycogen concentration reported here for unoperated goldfish liver is much higher than that found by Stimpson[14] (4.32 ± 0.54 mg/100 mg for 2 to 8 g fish). This may be due to the different method of analysis. Stimpson[14] used one of two methods (unspecified) described by Van der Vies[15]. It should be noted in comparing our results for unoperated fish with others in the literature that some difference may be attributable to maintaining unoperated fish in dilute saline for 4 to 5 weeks.

Protein determination by the method of Lowry et al.[10] gave consistently lower values than the biuret method[11]. The difference between the 2 methods was significant for sham operated and unoperated fish, but not for hypophysectomized fish. The protein concentration reported here for unoperated goldfish liver is higher than that reported by Hopper[16] (56.8 ± 3.0 mg/g for 29 to 59 g fish) but similar to concentration calculated by Das[17] (8.4 mg/100 mg for 14 to 18 g fish held at 25 °C).

Das[17] also determined the water content of goldfish liver and found it to be 72.3% ± 0.69%, slightly higher than the 68.6% reported here. Stimpson's[14] determination of liver lipid (2.97 ± 0.26 mg/100 mg tissue, wet wt.) is similar to our finding.

It is apparent that the approximate doubling of liver mass following hypophysectomy is a result both of glycogen, which increases absolutely and relatively with respect to controls, and of water, which increases to maintain a constant proportion. The decrease in lipid and protein concentrations can be accounted for by dilution with doubling of liver size, i.e. the absolute amounts of lipid and protein are very similar in unoperated, sham-operated, and hypophysectomized goldfish. This indicates that there is no elaboration of liver tissue but an accumulation of glycogen into pre-existing hepatocytes with the concomitant addition of water, until the glycogen content of hypophysectomized goldfish livers is approximately 2.5 times that of controls. This is in contrast to most other vertebrates where, after hypophysectomy, liver weight decreases and glycogen content remains constant or decreases[18].

Summary. The increase in the size of goldfish (*Carassius auratus*) liver following hypophysectomy is due to a net accumulation of glycogen and the addition of water to maintain a constant proportion with no change in the absolute amount of either protein or lipid.

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