adenine sulphate, 100; nicotinic acid, 5·0; thiamin HCl, 1·0; pyridoxin HCl, 1·0; sucrose, 30000 and Difco-Bacto agar, 8000. The cultures were incubated at a light intensity of 3000–3200 lux with 16 hours daily light period at a temperature of 27 ± 1° C.

The callus cultures differentiated into leafy shoots and roots on media containing varying auxin/cytokinin concentrations. High kinetin/NAA ratio (2·0/0·2 mg/l) was found to be favourable for differentiation of callus cultures into leafy shoots (36%), whereas low kinetin/NAA ratio (0·2/0·5 mg/l) was responsible for root differentiation (89·4%). Higher NAA in the medium resulted in decreased root differentiation and the differentiated roots again started de-differentiation on coming into contact with the medium. Kinetin is not essential for root differentiation but its presence in the medium was observed to be critical for root growth. The callus cultures were found to differentiate into leafy shoots with one to four growing points. The differentiated leafy shoots could be made to form roots on a simple medium devoid of auxin (Fig. 1). The cultures once differentiated into roots first could not be made to form shoots and the root growth continued for a long time. Hence, complete papaya plants could be regenerated through *in vitro* culture of stem segments by following a sequence of 3 stages. The first stage is concerned with the establishment of prolific callus growth, in the second stage the callus differentiates into leafy shoots and, the growing leafy shoots are made to form roots in the third stage. In certain plant species, it is possible to proceed with the first two or sometimes three stages by using the same culture medium, but in papaya the requirements of each stage has been observed to be specific.

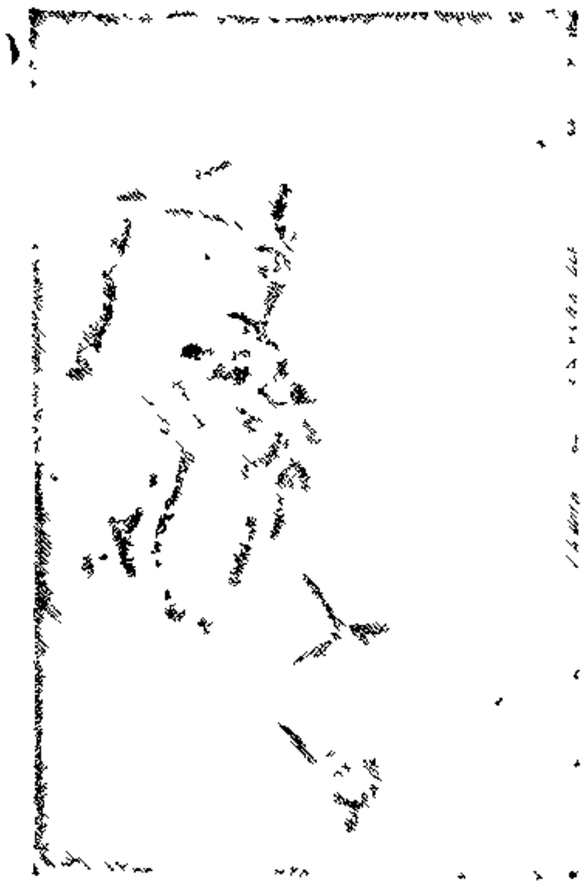

FIG. 1. Papaya plantlet 12 weeks after inoculation on a medium containing 2·0 mg/l kinetin and 1·0 mg/l NAA and then sub-culturing it on a medium devoid of auxin (× 1·7).

This technique can be employed for the proliferation of tissue obtained from mature papaya plants for the commercial multiplication of clones of desired genotype and sex.

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CADMIUM INTOXICATION ON TISSUE GLYCOGEN CONTENT IN THREE FRESH WATER TELEOSTS

THOUGH several studies appeared in the past regarding the effects of environmental pollutants on aquatic organisms, they pertain to mortality studies. The reports on the damage caused to different internal organs and the changes in various physiological and biochemical processes and causes of death after exposure to aquatic poisons are relatively few[1]. Contamination of water and the utilisation of cadmium in industries cause health hazards of considerable magnitude. Cadmium intoxication is responsible for hypertension, renal tubule damage, emphysema, liver dysfunction, cancer[2] and exhibits a marked tendency to accumulate in the body with a very long biological half life[3].

The aim of the present investigation is to provide information on the internal disturbances of physiological and biochemical nature caused by cadmium intoxication. The author has studied the effects of cadmium intoxication on glycogen content in liver, muscle, brain, and kidney tissues in *Labeo rohita* (Ham), *Ophicephalus punctatus* (Bloch) and *Clarias batrachus* (Linn.).

Fifteen live *L. rohita*, *O. punctatus* and *C. batrachus* 18–20 cm in length were obtained locally and acclimatized in the laboratory for 4 days; 5 fishes of *L. rohita*, *O. punctatus* and *C. batrachus* were kept in the glass aquarium containing (5, 10 and 15) × 10^{-4}% of cadmium nitrate for 3 hours. Liver, muscle, brain and kidney tissues were removed after decapitation and dissection of the treated fishes. The tissues were soaked on a filter paper in order to remove the adheing fluid. The preparation of tissue samples, estimation

TABLE I

Variations in Tissue Glycogen Content in a Fresh water Major Carp, Murrel and Cat Fish due to Cadmium Intoxication

Name of the tissue	Control	Glycogen (μ gm/gm wet weight of the tissue)		
	(Cadmium vol. %)	(5×10^{-4})	(10×10^{-4})	(15×10^{-4})
L. rohita				
Liver	4248·8±260·4	3028·5±164·4	2456·5±69·5	1596·4±96·9
Muscle	3085·3±195·2	21944·±96·4	1094·3±76·4	696·5±48·5
Brain	1076·2±58·4	1210·4±70·5	1398·4±105·7	548·5±32·4
Kidney	708·3±24·2	908·9±36·2	1024·4±80·9	328·0±18·9
O. punctatus				
Liver	3158·1±105·4	2236·4±155·3	1850·4±90·8	996·5±40·4
Muscle	2264·4±285·0	1438·2±132·5	856·2±50·3	609·2±24·3
Braine	902·1±41·5	1025·2±95·6	1165·4±25·4	505·4±18·9
Kidney	589·5±46·5	726·1±32·4	798·5±36·4	274·5±16·3
C. batrachus				
Liver	2806·6±165·8	2146·5±95·3	1905·1±62·4	905·5±54·4
Muscle	1760·2±120·3	1218·9±70·2	806·4±34·5	425·5±31·4
Brain	720·8±67·4	858·4±48·5	976·8±76·5	369·4±11·1
Kidney	484·3±59·4	628·1±31·5	710·5±26·3	240·5±12·4

Values are mean ± SE of 5 replicates.

and expression of glycogen values are described elsewhere[4]. The experiments were repeated 5 times to subject the data to statistical analysis. Glycogen content of the above mentioned tissues of untreated fishes was taken as control.

Cadmium nitrate intoxication has brought about a number of significant changes in the glycogen content of liver, muscle, brain and kidney tissues of *L. rohita* *O. punctatus* and *C. batrachus* (Table I). No visible symptoms of toxic reaction was seen in the fishes exposed to 5, 10, and 15×10^{-4}% of cadmium for 3 hours. One of the prominent effects of cadmium on *L. rohita*, *O. punctatus* and *C. batrachus* was the great reduction in the size of the liver. The percentage of fall happens to be in *L. rohita* 62, 77, 40, 54%, in *O. punctatus* 68, 73, 44, 53% and in *C. batrachus* 68, 76, 57 and 50% in liver, muscle, brain and kidney tissues respectively. In liver and muscle the author observed an inverse relationship between the concentration of cadmium and the fall in the content of glycogen. Whereas in brain and kidney the glycogen level increases upto 10×10^{-4}% of cadmium and at 15×10^{-4}% the process of glycogenolysis was maximum in all the three fishes.

It has been already reported that acute cadmium poisoning damages the tissues in fishes[1,5]. Reduction in the size of the liver and tissue damage may be responsible for glycogen depletion and further, it might have affected the capacity of liver to store glycogen in *L. rohita*, *O. punctatus* and *C. batrachus*. It seems that cadmium accumulates in the pancreatic islets of the fish and selectively damages the insulin producing B-Cells[6]. The fall in the content of muscle agrees well with the impaired secretion of insulin.

The kidney is the main sequestering organ for cadmium and disturbed renal function is a common sign of short and long term cadmium poisoning in vertebrates[1-2]. The increase in the glycogen content at 10×10^{-4} % of cadmium exposure may be associated with increased levels of glucose. At 15×10^{-4}% of cadmium the depletion in renal glycogen content is maximum. Cadmium might have damaged the renal tissue and the fall in glycogen content may be related with the above phenomena. In brain tissue, it looks difficult to predict the exact mechanism of rise and fall in tissue glycogen content by cadmium intoxication at lower (5×10^{-4}%) and (at higher (15×10^{-4}% concentrations.

It is well established that cadmium has a high affinity for sulphydryl and hydroxyl groups and ligands containing nitrogen[7]. Thus binding to such groups in chemical system might affect various basic biochemical and physiological processes and thereby interfere with the central functions of the organism even at very low cadmium concentrations. The present results on tissue glycogen content in *L. rohita*, *O. punctatus* and *C. batrachus* indicate that cadmium in the water might produce dysfunctions of several physiological and biochemical processes in fish and such a mechanism may be responsible for the differential effect of cadmium on tissue glycogen content.

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PRODUCTION OF DIOSGENIN FROM *COSTUS SPECIOSUS* (KOEN) SM., AND *SOLANUM NIGRUM* L., SUSPENSION CULTURES

DIOSGENIN, an important sapogenin from economic point of view, has been reported from tissue cultures of several plant species[1-7]. Previous work on *Costus speciosus* and *Solanum nigrum* from *in vivo* and *in vitro* has been described[5-13] and in this communication we wish to report our findings on production and isolation of diosgenin from suspension cultures of these plant species.

Seedling calli of *C. speciosus*[7] and *S. nigrum* (hexaploid[5,6]) maintained for 18 months as static cultures were transferred in RT liquid medium supplemented with 0·1 ppm of 2,4-dichlorophenoxyacetic acid and grown as suspension cultures for 6–8 months by frequent subculturings of 4–6 weeks. The cultures of *C. speciosus* showed root formation, so callus was transferred to RT liquid medium supplemented with 1 ppm of 2,4–D, which resulted undifferentiated cells.

Suspension cultures of *C. speciosus* (differentiated) and *S. nigrum* were transferred to fresh liquid medium with 0·1 ppm of 2,4–D and allowed to grow for different time intervals of 2, 4 and 6 weeks. All the tissue samples were harvested separately, dried and growth indices calculated (GI = Final dry weight of tissue — Initial dry weight of tissue/Initial dry weight of tissue). Each of the dried tissue samples was powdered and extracted for its steroidal content[7]. Diosgenin was quantitatively estimated following a spectrophorometric method using thin layer chromatography[7], Absorbances were read on a Spectrophotometer (Carl Zeiss, JENA, DDR, VSU–2P) at 405 nm. Ten replicates were performed in each case and the mean values were taken.

Maximum growth index (6·8; 6·1) was observed in six and four weeks old suspenison cultures of *S. nigrum* and *C. speciosus* respectively.

Diosgenin was confirmed by its mp (204–206° C), mmp (203–204° C), Co-chromatography [TLC, Silica gel acctone benzene = 1 : 2 (R_f 0·71), chloroform–acetone = 8 : 2 (R 0·57); anisaldehyde and 50% H_2SO_4 as spraying reagent] and identical IR spectral studies.

Diosgenin content was low (0·15%) in differenciated cultures of *C. specicsus* (Table I) when compared with undifferentiated cells as suspension cultures (0·48%)[7] which supports the previous findings of Kaul and Staba[4] who have also found higher amount of diosgenin in undifferentiated suspension cultures of *D. deltoidea* as compared with differentiated liquid cultures. However in *S. nigrum* suspension cultures, maximum diosgenin content (0·20%) was noted in six weeks old cultures (Table I) which was low when compared with its static cultures (0·65%)[6].

TABLE I

Growth indices (GI) and diosgenin content in Costus speciosus and Solanum nigrum *suspension cultures*

Age of tissue (weeks)	*C. speciosus* (differentiated)		*S. nigrum*	
	GI	Diosgenin (%)	GI	Diosgenin (%)
2	2·8	0·036	3·5	0·143
4	5·3	0·10	5·5	0·174
6	3·4	0·15	6·8	0·200