SHORT COMMUNICATIONS

EFFECT OF GIBBERELLIC ACID ON GROWTH AND ALKALOIDS IN HYOSCYAMUS MUTICUS LINN

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Introduction

Attempts have been made by various workers to investigate the effects of growth hormones on various economic plants[1] but very little work has been carried out on medicinal plants. Gibberellic Acid (G.A.) is a growth regulator and its obvious effect on plants is to accelerate vegetative growth of the shoot. It has also been employed by various workers to increase physical size of plants. In the present study, Hyoscyamus muticus was selected to study the effect of G.A. on vegetative growth as well as on its alkaloid biogenesis.

Experimental

The seeds of Hyoscyamus muticus were germinated and the seedlings were raised in earthen pots and transplanted in beds after few weeks. A stock solution of G.A. (0.125 g.) in ethyl alcohol (125 ml.) was diluted with water just before use according to the following concentrations for spraying on two sets of plants, agral being used as wetting agent at the rate of 0.1 ml. per litre-

First set of plant – G.A. Conc. 50 p.p.m.
Second set of plant – G.A. Conc. 100 p.p.m.

To a third set of plants serving as control, only water with agral was sprayed. Spraying was done with the help of a hand atomiser.

Originally, spraying of the G.A. was done when the plants were six-week old. The subsequent spraying was done after an interval of six weeks.

Growth Observations.—The plants responded very markedly to the spray of G.A. soluction. Within a few days, the rosette habit of the plants was broken and these were found considerably larger than the untreated ones. In consequence, the internodes of the treated plants grew longer than the control plants.

The average size and rate of growth of the treated plants was maximum with 50 p.p.m. of G.A. but decreased with the increase in concentration of G.A.

Early flowering was also induced in the treated plants. The first flower appeared in the plants sprayed with 50 p.p.m. of G.A. The physical yield was maximum in the plants sprayed with 50 p.p.m. G.A. solution, followed by 100 p.p.m. G.A. The increase of about 20% in the 50 p.p.m. treated plants was more as compared to the control plants.

Chemical Assay.—Leaves of the mature plants were collected for the estimation of total alkaloids and dried at 40°C. in the oven. The B.P.[3] method was adopted for the chemical assay. Three assays were performed for each of the treated, as well as control plants. Following are the observations of the percentage of total alkaloids:

Sr. No.	Sample	Percentage of total alkaloids
1.	Control plants	0.326
2.	50 p.p.m. G.A.	0.209
3.	100 p.p.m. G.A.	0.200

Conclusions

From the above, it will be seen that G.A. spray has tended to increase the physical size of the plants by enlarging the internodal part of the stem.

The results of the chemical assay, show that the amount of total alkaloids has decreased. Although G.A. has increased the physical yield of the Hyoscyamus muticus plants but the corresponding decrease in the alkaloids rendered it uneconomical for use on an extensive scale.

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NON-SPECIFIC INHIBITION OF THE RAT UTERUS BY HEPARIN

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It has been reported by Keller[1] that heparin antagonises the actions of 5-hydroxytryptamine (5-HT) on isolated rat colon and rat uterus. A concentration of 0.25 to 0.5 mg./ml. of heparin was necessary to bring about this effect. Keller further claimed that this antagonism between 5-HT and heparin can also take place *in vivo*. He has shown that 5-HT and tryptamine, only in high doses, "reduces the antithrombin time prolonged by the action of heparin". Smith and Smith[2] further showed that 5-HT injected into heparinised animals loses it's vascular depressant effect.

In the work reported here, an attempt is made to study this phenomenon in detail. It was noted that heparin not only inhibits the response of the rat uterus to 5-HT, but also that to oxytocin, acetylcholine and potassium. However, no such antagonism could be demonstrated *in vivo*, on cat blood pressure and rat uterus *in situ*.

Materials and Methods

1. Superfused Rat Uterus.—Contractions of the isolated rat uterus were recorded by the method of Gaddum.[3] One horn of the rat uterus was suspended in an organ bath; one end being connected to a frontal writing lever. de Jalon's solution, heated upto 27-28°C. was allowed to drip on the tissue at a constant rate of 60 drops per minute.

Test solution, also warmed upto 27-28°C. were applied to the uterus at intervals after stopping de Jalon's solution. Thirty seconds after, superfusion was started again. The volume of the test solutions applied to the uterus was kept constant.

Solutions of oxytocin (0.1 mU/ml.), acetylcholine (1.0 µg./ml.) and potassium chloride (10 µg./ml.) were applied to the surface of the rat uterus. The contractions obtained were compared with those obtained after adding 0.5 to 5.0 mg./ml. heparin in the test solutions. In all experiments solutions containing no heparin were much more active than those with heparin. As shown in Table 1, the contractions were reduced by 75 percent in the presence of heparin in case of oxytocin and acetylcholine; while the contractions produced by potassium chloride were not significantly reduced until the concentration of heparin was increased to, between 2.5 and 5.0 mg./ml. The result of a typical experiment is shown in Fig. 1.

TABLE 1.

	Without Heparin	With Heparin
1. Oxytocin	84,107,65,74	20,38,15,10
2. Acetylcholine	90,82,69,50	15,10, 6,10
3. Potassium	101,91,83,62	62,80,60,47

Superfused rat uterus. Figures denote the height of contractions in mm. Contractions without heparin are significantly larger than those with heparin in a concentration of 0.5 to .5 mg. 1ml.

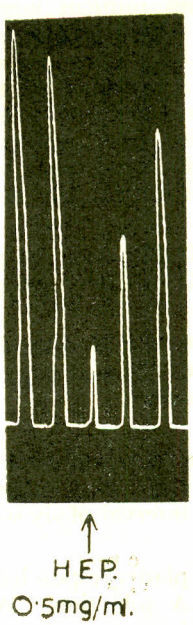

Fig. 1.—Isolated superfused rat uterus. Contractions were caused by applying 0.5 ml. oxytocin diluted in de Jalon's solution (0. 1 MU/ml). At the arrow the same dose of oxytocin was applied, but it was diluted in de Jalon's solution containing 50 i.u. heparin per ml.

2. Rat Uterus in situ.—A young virgin female rat in oestrous was anaesthetised with urethane (0.7 ml. of 25% solution of urethane per 100g. body weight) and was fixed prone on a dissecting table. Abdomen was opened and one horn of the uterus was dissected free from the surrounding tissues and its ovarian end attached to a hook which was in turn connected to the frontal writing lever. The uterus was kept moist by de Jalon's solution warmed upto the body temperature. Drugs were injected via a cannulated jugular vein while the contractions of the uterine horn were recorded on a smoked drum.

Intravenous injections of 5 mU of oxytocin caused the uterus to contract (Fig. 2). However, the contractions produced when oxytocin was combined with heparin, sufficient to produce a concentration in the blood of 0.7 mg./ml., were no smaller than the control contractions.

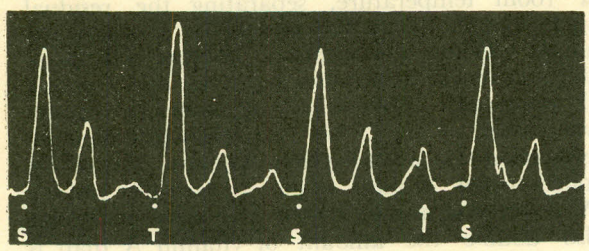

Fig. 2.—Response of rat uterus *in situ* to intravenous injections of oxytocin. S. Oxytocin 5 mU. T. oxytocin 5 mU plus heparin 2 mg. At the arrow 5 mg. heparin was injected intravenously but the response to oxytocin given 2 min. later was not reduced.

3. Cat Blood Pressure.—Blood pressure was recorded from the carotid artery of the cat under chloralose anaesthesia (80 mg. chloralose/kg. body weight). Injections of suitable doses of acetylcholine, 5-HT, oxytocin and vasopressin were made into the cannulated femoral vein. The doses were repeated ten minutes and one hour after intravenous injection of 10,000 i.u. of heparin.

No significant difference was noticed in the blood pressure responses after heparin (Fig. 3).

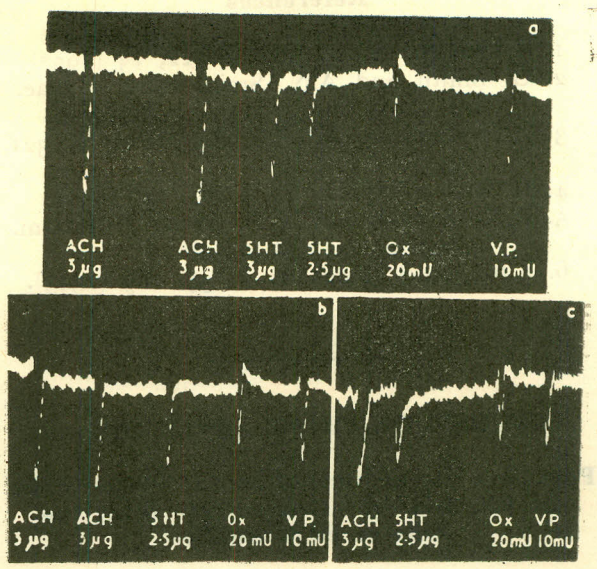

Fig. 3.—Blood pressure recording from a cat under chloralose. Effect of intravenous injections of acetylcholine (Ach), 5-hydroxytryptamine (5-HT), Oxytocin (Ox), and Vasopressin (VP). Time interval between a and b is ten minutes and between a and c, one hour. Between a and b, 100 mg. heparin was injected intravenously.

Results and Discussion

It has been claimed that heparin antagonizes the action of 5-HT both *in vitro* on the rat uterus,[4] and *in vivo* on the cat blood pressure.[2] It is apparent from the present study that heparin not only antagonizes the action of 5-HT on the rat uterus but also that of substances of such widely different chemical structure as acetylcholine, oxytocin and potassium chloride This nonspecific inhibitory action is therefore, probably due to the occupation by heparin of receptors in the muscle and not, as Keller suggested, to its combining with and thus inactivating the molecule of 5-HT. Further proof in favour of this conception is obtained from the work of Sanyal and West,[5] who have shown that heparin, when incubated *in vitro* with 5-HT, adrenaline, noradrenaline and histamine, forms complexes only with histamine and does not combine in any way with any other of the above mentioned substances. The inhibition of the uterus occurs only *in vitro*, since heparin does not affect the contractions of the rat uterus *in situ* caused by the intravenous injections of oxytocin, even when the concentration of heparin in blood reaches about 0.7 mg./ml. The findings of Smith and Smith[2] could not be confirmed on cat blood pressure. Heparin even in amounts four times the normal doses did not antagonize the actions of acetylcholine, 5-HT, oxytocin or vasopressin. Since heparin becomes attached to the molecules of plasma proteins when it is injected intravenously,[6] it is possible that it is not available in sufficient amounts to be able to antagonise the actions of the above mentioned substances.

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STUDIES ON SHARK LIVER OIL

Part III.—Use of Alcohol for the Preparation of Vitamin "A" Concentrate from the Oils of High Acidity

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Introduction

Vitamin A and D concentrates are generally prepared in two stages: (i) By extracting them from the fish liver and body oils with a solvent which should be completely miscible with the oil above room temperature but sparingly at lower temperatures (ii) Purification of semi concentrate by treatment with a highly polar solvent containing at least 9 percent water. Solvents of the first group are aliphatic and alicyclic monohydric alcohols containing 3-6 carbon atoms. In the second group of solvents, methyl alcohol and 85-90 percent aqueous propanol have been used by Loran and Buxton,[1] for the preparation of the concentrate.

In the present investigation use has been made of ethyl alcohol for preparing vitamin A concentrate from commercial shark liver oil. This oil is prepared by primitive methods and sold in the local market for smearing fishing boats. Although this oil contains an appreciable amount of free fatty acids and produces rancid smell, it is very rich in vitamin A. Using this oil as the starting material the authors have attempted to separate the free fatty acids on the one hand and to prepare vitamin A concentrate on the other. The residual oil after the separation of vitamin A extract was devoid of undesirable fishy smell and taste and contained 80-85 percent vitamin A to enable it to serve as standard medicament.

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Experimental

The crude oil was obtained from the local market. It was dark brown in colour, produced rancid smell, contained 5000-8000 I.U. vitamin "A"/g. and 1.2 percent acidity.

In the present investigation 500 g. of the oil was treated with 500 ml. alcohol on a boiling water bath, or shaken with the same amount of alcohol at room temperature, separating the residual oil I from the alcoholic extract, in a separating funnel. When the alcoholic extract was kept at room temperature overnight an oily fraction II separated. The remaining alcoholic extract was chilled at -15°C. for two hours and after the separation of a whitish yellow fraction III, it was concentrated *in vacuo* to give a dark oily residue IV containing almost all the free fatty acids and also double the amount of vitamin A than is present in the starting material.

The details of the experiment are given in Tables 1 and 2.

Thinning of the raw oil with petroleum ether and subsequent extraction of the vitamin A with alcohol was also tried, and it was found that 88 percent of the oil and 81 percent of vitamin A remains in petroleum ether. It was, therefore, concluded that preparation of the vitamin A concentrate with alcohol at room temperature would be a more industrially feasible proposition.

Preparation of Vitamin A Concentrates from Fraction IV.—The alcoholic fraction IV as separated above was saponified and vitamin A extracted with ether. The ether extract was distilled off in an atmosphere of nitrogen and the last traces were removed under *vacuo*. The potency of such concentrates was found to be about 10 times the alcoholic fraction IV and 20 times that of the original oil. A method was subsequently developed for the simultaneous refining of shark liver oil and preparation of vitamin A concentrate which is described elsewhere.[9]

TABLE 1.

Fraction No.	Number of extracts	Weight in g.		Percent of original crude oil	Percent free fatty acids	Vitamin A I.U. per g.	Average vitamin A/g. in each fraction
1.	—	440		88.0	0.18	6000	6000
	1st	8.01		1.6	0.80	6500	=6408
2.	2nd	7.50		1.5	0.49	6450	
			=27.99				
	3rd	6.47		1.3	0.48	6300	
	4th	6.01		1.2	0.48	6340	
3.	1st	2.02		0.406	1.6	6280	
	2nd	1.64		0.328	1.6	6400	
			=6.98				=6571
	3rd	1.78		0.356	1.3	6520	
	4th	1.53		0.306	1.3	6561	
4.	1st	9.44		1.9	19.0	10926	
	2nd	6.64		1.3	14.7	12025	=12231
			=24.86				
	3rd	4.98		1.0	10.5	13758	
	4th	3.80		0.8	9.4	13829	13829

Wt. of the oil fractionated =500 g.; Vitamin A per g. of oil =7000 I.U; Free fatty acids as oleic acid =1.2%.

TABLE 2.—SHOWING THE FRACTIONATION OF CRUDE SHARK LIVER OIL WITH ETHYL ALCOHOL AT ROOM TEMPERATURE.

	Fraction I	Fraction II	Fraction III	Fraction IV
1. Wt. of fraction in g. by difference	446.40	4.22	5.88	23.50
2. Percent of crude oil	93.28	0.845	1.175	4.70
3. Percent of free fatty acids as oleic acid	0.15	0.5	1.30	16.0
4. Vitamin A I.U./per g.	6000	5800	5900	13,000

Discussion

Commercial shark liver oil, which is available in the local market, is characterised by a strong fishy odour containing free fatty acids upto 1.5 percent. Refining of this oil by the conventional methods, entail appreciable loss of vitamin A and of neutral oil. In dealing with oils of this type, it has been found advantageous to remove odoriferous matter, free fatty acids and other impurities along with vitamin A by extraction with a solvent which is least miscible with oil. In the present investigation alcohol was preferred to solvents such as methyl and butyl alcohol, xylene, toluene, and fusel oil used by previous workers[2–6] because ethyl alcohol is produced in the country.

It can be seen from Table 1 that fraction II is almost pure oil but III is a mixture of oil and fatty acids. Higher percentage of acidity in III as compared to II shows some separation of free fatty acids on chilling. The last fraction IV is twice as rich in vitamin A as the original oil and contains most of the free fatty acids. The residual oil I which contains very little amounts of free fatty acids retains 80-85 percent of vitamin A of the original oil and can be used for medicinal purposes.

The usual methods for the preparation of vitamin A concentrates consists in the saponification of the total oil and recovery of vitamin A concentrates from the non-saponifiable fraction. A comparatively more recent development in this direction is based on the molecular distillation of the liver oils. In either of these methods the bulk of the oil is not available for therapeutic use, and the bye-products obtained in these processes cannot, in consequence, be considered as having been utilized to their full economic advantage. If, however, the crude liver oil is first extracted with organic solvents such as ethyl alcohol, and stearin is then separated from the residual oil I concentrates of vitamin A can be prepared from the stearin fraction as well as from the organic solvent of the oil. Moreover the purified stearin thus obtained, can also be used for the manufacture of shark liver oil emulsions, syrup etc. Since, the solvent extraction removes odoriferous matter, free fatty acids and other impurities, deodorisation of the oil with superheated steam is obviated.

Interest in continuous solvent extraction of vegetable and animal oil has been shown in comparatively recent years with the advent of commercial extractors which are designed to process ordinarily two liquids on the counter current principle. Since these methods of solvent fractionation are based on solvents like furfural and liquid propane (both imported items), the present process based on indigenously produced alcohol could be independent of the need of importing.

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EXTRACTION OF URANYL SALTS BY THENOYLTRIFLUOROACETONE IN CARBON TETRACHLORIDE

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Introduction

2-thenoyltrifluoracetone (TTA) has been used by many workers[1–6] as a chelating agent in the separations of various metals by solvent extraction. Day and Powers[7] investigated the extraction of uranyl ion with TTA in benzene from the aqueous solution of sodium salts of perchlorate, chloride, nitrate, sulphate and fluoride. They reported the stability constants of uranyl ion by the anion of the various salts. Walton *et al.*[8] have carried out studies on the extraction of uranyl ion from nitric acid and discussed the theoretical aspects of the extraction. Hyde[9] mentioned that the extraction of uranyl ion from lithium perchlorate-perchloric acid is very low whereas the extraction from nitrate medium with polar solvents is comparatively high. The preliminary studies of Hyde and Tolmach[10] indicated that the separation of thorium and uranium may be possible by extraction with TTA in benzene. Recently Irving and

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Edgington[11] observed the synergic effect in the solvent extraction of uranium (VI) with TTA and tri-n-butyl phosphate. The present work was undertaken to study the extractibility of uranyl ion from nitric, hydrochloric, and sulphuric acids media with TTA in carbon tetrachloride. Carbon tetrachloride is chosen as being more advantageous than benzene, being non-inflamable, relatively stable, easily available in pure state, less soluble in water, and having lower dielectric constant. Both uncharged and charged complexes of uranyl ion are formed with TTA of which the former one only is soluble in organic solvent. The distribution ratio has been expressed in this work as,

$$Ku = \frac{[U]\ org.}{[U]\ aq.}$$

where the subscripts org. and aq. refer to the organic and aqueous phase respectively.

Experimental

Reagents: The reagents used were A.R grades uranyl nitrate, TTA, carbon tetrachloride, nitric, hydrochloric and sulphuric acids, and zinc amalgam. (The manufacturers were B.D.H. or E. Merck.) Uranyl chloride was prepared by dissolving metallic uranium in hydrochloric acid and carefully oxidizing with a small amount of nitric acid and then evaporating to dryness. Uranyl sulphate was prepared from uranyl nitrate by fuming twice with concentrated sulphuric acid and evaporating to dryness. The distribution ratio have been determined by extracting 10 or 20 ml. of uranyl solution with equal volume of TTA in carbon tetrachloride for 30 minutes in 250 ml. separatory funnel at room temperature. The phases were separated and the uranium content of both aqueous and organic phases were determined by volumetric method of Rodden and Wharf.[12]

Results and Discussion

The extraction as a function of acid concentration at different uranium loading and at constant TTA concentration is investigated. The results in hydrochloric, sulphuric, and nitric acid are shown in Table 1. The values of Ku at higher acid concentration are somewhat irregular. This may be due to the formation of different unstable species of varying composition. The distribution ratios in very low acid concentration of hydrochloric and sulphuric acid are higher than those in nitric acid.

TABLE 1.—DISTRIBUTION RATIO OF URANYL CHLORIDE, SULPHATE AND NITRATE IN VARYING NITRIC ACID CONCENTRATION AND, URANIUM LOADING AT CONSTANT 0.20 M TTA.

Normality of acid	Uranium in M	Ku		
		HCl	H_2SO_4	HNO_3
4.000	0.0042	0.010	0.030	0.040
0.500	0.0042	0.099	0.044	0.030
0.050	0.0042	0.340	0.200	0.062
0.001	0.0042	13.120	14.140	2.850
4.000	0.0084	0.054	0.010	0.021
0.500	0.0084	0.130	0.120	0.042
0.050	0.0084	0.300	0.200	0.070
0.001	0.0084	4.300	5.580	2.730
4.000	0.0168	0.001	0.090	0.022
0.500	0.0168	0.006	0.100	0.051
0.050	0.0168	0.084	0.170	0.075
0.001	0.0168	1.390	1.600	1.480

The investigation on the TTA—CCl_4 extraction system in the three different acids were directed primarily to study the comparative suitability and optimum distribution in organic phase from the particular acid medium.

The extraction as a function of TTA of uranyl nitrate at constant 0.001 N nitric acid has been studied. The results are shown in the Table 2.

TABLE 2.—DISTRIBUTION RATIO OF URANYL NITRATE WITH VARYING TTA FROM 0.001 N HNO_3 SOLUTIONS.

TTA in M	Uranium in M	Ku
1.00	0.0042	8.18
0.50	,,	3.85
0.10	,,	1.40
0.02	,,	0.20
1.00	0.0084	6.36
0.50	,,	2.85
0.10	,,	1.08
0.02	,,	0.15

The percentage extraction of 0.0042 M uranyl ions with 0.2 M TTA from different acids media of concentration 0.001 N have been calculated after Morrison and Freiser.[13] In a single batch extraction of uranyl chloride, sulphate, and nitrate, an extraction of 92.9 percent, 93.4 percent and 74.02 percent respectively, have been obtained.

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