nitrobenzyl bromide (0.1 mol) in dry ether was added, under stirring to an appropriate substituted aniline (0.01 mol). The reaction mixture was stirred for about 9 hr, the ether layer was washed with cold water, and the separated solid was recrystallised from ethanol. IR:3120(NH), 1530, 1340 (NO_2) for all the compounds. The compounds were obtained in about 45–50 % yield.

1[N-Substituted-N-(4'-dimethylamino-3'nitro benzyl)amino]-2-[4''-(imidazolin-2 yl amino) benzoyl]-ethane hydroiodides (VI): Appropriate N-substituted aryl-3-nitro-4-N,N-dimethylamine benzylamine (0.025 mol) in ethanol (20 ml) was mixed with aq. formaldehyde solution (40 %, 1 ml) and compound (VI), (0.025 mol). The reaction mixture was heated for 10 min on a steam bath, left for 24 hr, washed with pet. ether (60–80) and recrystallised from ethanol. The physical data of these compounds are cited in table 2 and the reaction scheme is shown in figure 1. The compounds were obtained in about 50–60 % yield. IR: 3350 (NH, 3050, 2880 (CH), 1660 (C=O), 1530, 1340 (NO_2) for all compounds. PMR: ($CDCl_3$) of compound (2), (chemical shift in ppm), 2.88 (s, 6H, N-$(CH_3)_2$; 6.70–7.50 (m, 10H, Ar-H); 8.02 (s, 1H, NH), 3.48 (s, 4H, CH_2-CH_2) etc.

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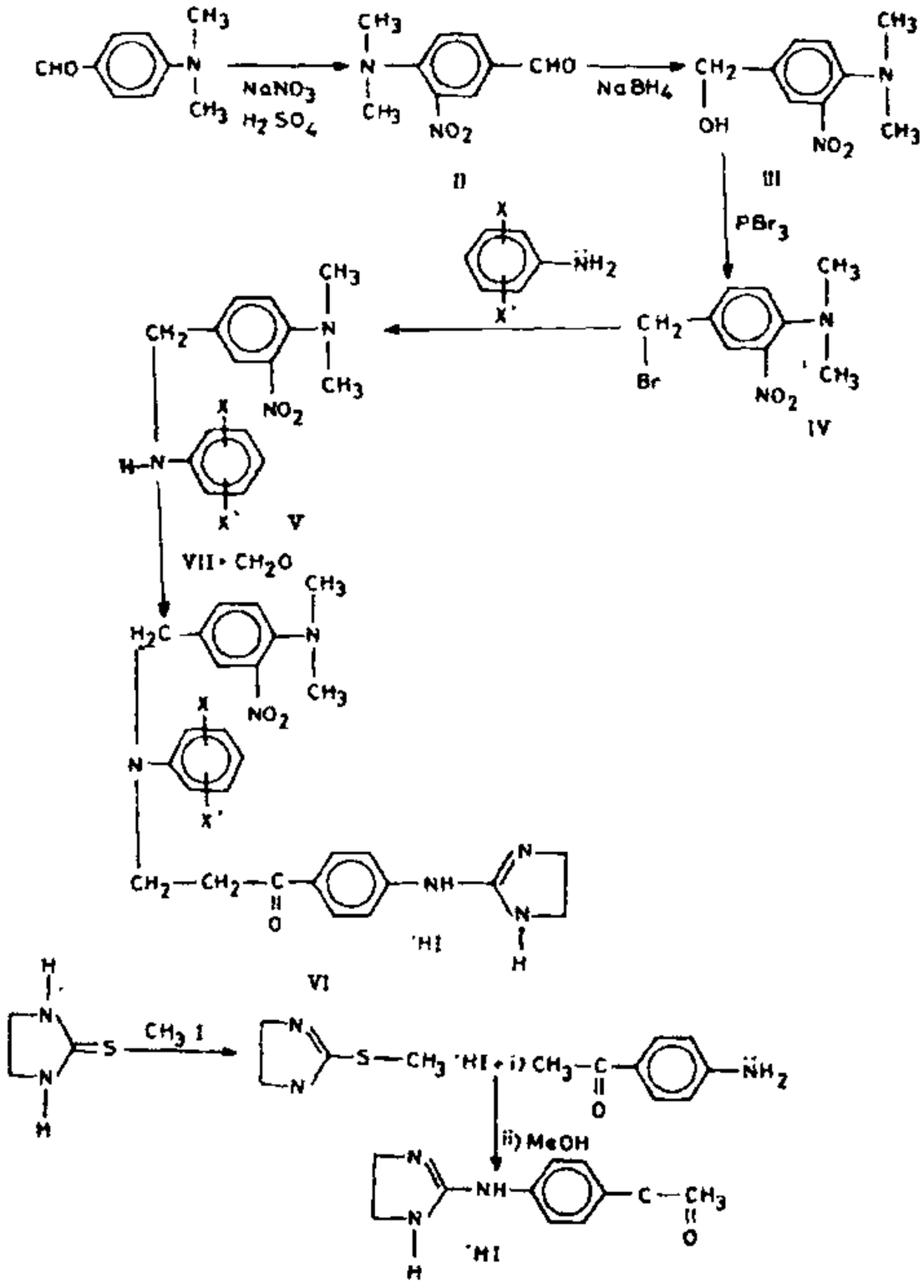

Scheme 1

A NEW RAPID COLORIMETRIC ESTIMATION OF UREA IN URINE USING ORTHOPHTHALALDEHYDE

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ORTHOPHTHALALDEHYDE (OPT) is a fluorogenic substance[1] that reacts nonspecifically with a number of biogenic compounds[2, 3], yielding intensely fluorescent condensation products in an alkaline medium in the presence of a reducing agent[7]. OPT was also used to detect peptides in fractions collected during column chromatography.

OPT is found to give a coloured product with diaminopropionic acid which is obtained on hydrolysis of β-N-oxalyl α, β-diaminopropionic acid (ODAP) in the *Lathyrus sativus* seeds.[4].

Other methods of urea estimation in urine generally used, include the diacetyl monoxime method[5] and the Berthelot method[6]. The present method is found to be sensitive, simple, rapid and versatile as it can also be adapted for enzymatic reactions in which ammonia evolves.

Chemicals

(a) Urease (BDH). One tablet hydrolyses 80 mg of urea at 15°C in 3 hr.
(b) Orthophthalaldehyde (Sigma Chemical Co. USA)
Other chemicals were of reagent grade or analytical reagent grade.

1. *Urea standard*:
Stock: 500 mg of urea is dissolved in 100 ml of phosphate buffer 0.1 M pH 7 and stored at 4°.
Working: 1 ml of stock standard is diluted 1:10 with glass distilled water whenever necessary.

2. *Urease*:
One tablet of urease is dissolved in 30 ml of 0.1 M phosphate buffer, pH 7 and centrifuged at 3000 g for 10 min at 4°.

3. *Orthophthalaldehyde*:
100 mg of OPT is dissolved in 0.2 ml mercaptoethanol and 1 ml 95% ethanol. The volume is made up to 100 ml with glass distilled water.

Samples of urine are collected and processed immediately. A 1:100 dilution is made with glass distilled water. The steps followed for the assay are as follows. Standard tubes are prepared using aliquots ranging from 50–250 μg. An aliquot of 0.5 ml is taken for the sample. The volume in all these tubes is made up to 1 ml with phosphate buffer 0.1 M pH 7. Subsequently 0.5 ml of urease is added in all the tubes followed by 0.5 ml of OPT reagent. A blank is prepared which has everything except sample. A control is prepared which has everything except urease. The difference in O.D. between the experimental tube and the control will give the value of urea. The tubes are incubated at 37°C for 15 min. The absorbances are read at 400 nm in a Beckman spectrophotometer.

Table 1 shows the values of absorbance for different concentrations ranging from 50–250 μg of urea. This relationship between concentration of urea and absorption at 400 nm is strictly linear.

Table 2 shows the quantitative determination of urea in normal samples of urine using the present method and the comparative values obtained using the commonly used diacetyl monoxime method.

Table 1 *Values of absorbance for different concentrations of urea ranging from 50–250 μg.*

Urea (μg)	O. D. at 400 nm
50	0.029
100	0.060
150	0.090
200	0.119
250	0.146

Table 2 *Values of normal urine samples using diacetyl monoxime and OPT methods expressed in grams per litre of urine*

Orthophthalaldehyde (OPT)	Diacetyl monoxime
22.3	22.6
25.3	26.0
25.6	26.5
30.6	31.6
25.0	25.6
35.3	36.1
28.7	28.0
31.0	29.9
26.0	25.1
38.0	38.4

This method is standardised for a number of conditions in the assay system. Various concentrations of urea were tried and the range that is found to be most satisfactory is 50–250 μg of urea. Different ranges of pH were used and the colour development was seen. The pH at which optimum colour is obtained is 7. The addition of OPT before incubation gave more consistent results than when it was added after incubation. The incubation time was varied over a wide range and it was found that 15′ is enough for maximum colour development. Similarly the optimum incubation temperature was found to be 37°C. The colour is stable for 1 hr. Similarly the OPT concentration when tried over a range gave optimal colour development at 0.5 mg/tube. The filters tried covered both the UV and the visible range and the most suitable wavelength is found to be 400 nm. The percentage recovery of the sample is found to be 98%.

Most amino acids give fluorescent products with OPT. Hence the effect of individual amino acids as also the normal constituents of urine like creatine, creatinine, oxalate, urate, citrate etc was seen by adding 10 mmol of these substances to the assay system. None of them is found to affect the development of the colour adversely.

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EFFECT OF PENITREM A ON GLUCOSE TOLERANCE STUDIED IN RATS

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PENITREM A, a tremorgenic mycotoxin is a secondary metabolite of *Penicillium crustosum*[1], which is a natural contaminant of corn, cheese and cotton seeds[2-4]. Penitrem A ingestion is found to increase blood glucose concentration[5]. Hence it was of interest to study the effect of penitrem A on liver glycogen concentration and serum insulin levels.

P. crustosum was grown on Czapek-Dox liquid medium enriched with 2% corn steep liquor for 15 days at 25°C. The toxin was extracted from the mycelial mats[6] and purified[7]. Penitrem A was recrystallised from ethyl alcohol. Modern bread (containing 0.2% calcium propionate as the preservative) was sterilized with 30% moisture content at 15 psi for 10 min, was inoculated (after cooling) with 5 ml spore suspension of *P. crustosum* containing 1×10^6 spores/ml and kept at 25°C for 15 days. The infected bread was treated with diethyl ether to kill the spores and the ether was removed completely. The diet when tested was found to be free from any fungal spores, but contained the toxin and was mixed with the commercial rat feed in the ratio of 1:2 and fed to rats, as "fungal contaminated diet" containing 5 to 8 ppm of penitrem A. The composition of the "contaminated diet", was similar to that of the control diet.

Young albino rats of Wistar strain weighing about 40–50 g were divided into 4 groups. One group of rats was fed with contaminated bread. Another group was injected intraperitoneally with 0.1 ml of pure penitrem A, dissolved in sesame oil (1 mg/kg body weight). Proper controls were used in each case. After 24 weeks of experimentation, blood was collected from the tail of the rats and blood glucose concentration was estimated[8]. Since the fasting blood glucose concentrations were elevated, tolerance to glucose was tested[9]. The rats were starved overnight and the fasting blood samples were collected. Ten per cent glucose solution (3.5 g per kg body weight) was administered orally. Blood samples were collected thereafter over a period of 180 min. One set of blood samples was collected with sodium fluoride as anticoagulant for glucose estimation and another set was collected for the assay of serum insulin according to the modified method of Herbert *et al*[10]. The animals were later killed and the liver glycogen[11] and total lipids[12] were estimated.

The results on GTT are presented in figures 1 and 2. In normal rats, maximum blood glucose concentration reached after 30 min of glucose load and it comes back to the fasting level after 180 min. But in the experimental rats, blood glucose concentration progressively increased after glucose load and the fasting level is not reduced after 180 min, indicating poor response to glucose load. Though insulin concentration increases immediately after glucose load in the experimental animals (table 1), this increase is insufficient to utilize the ingested glucose, indicating

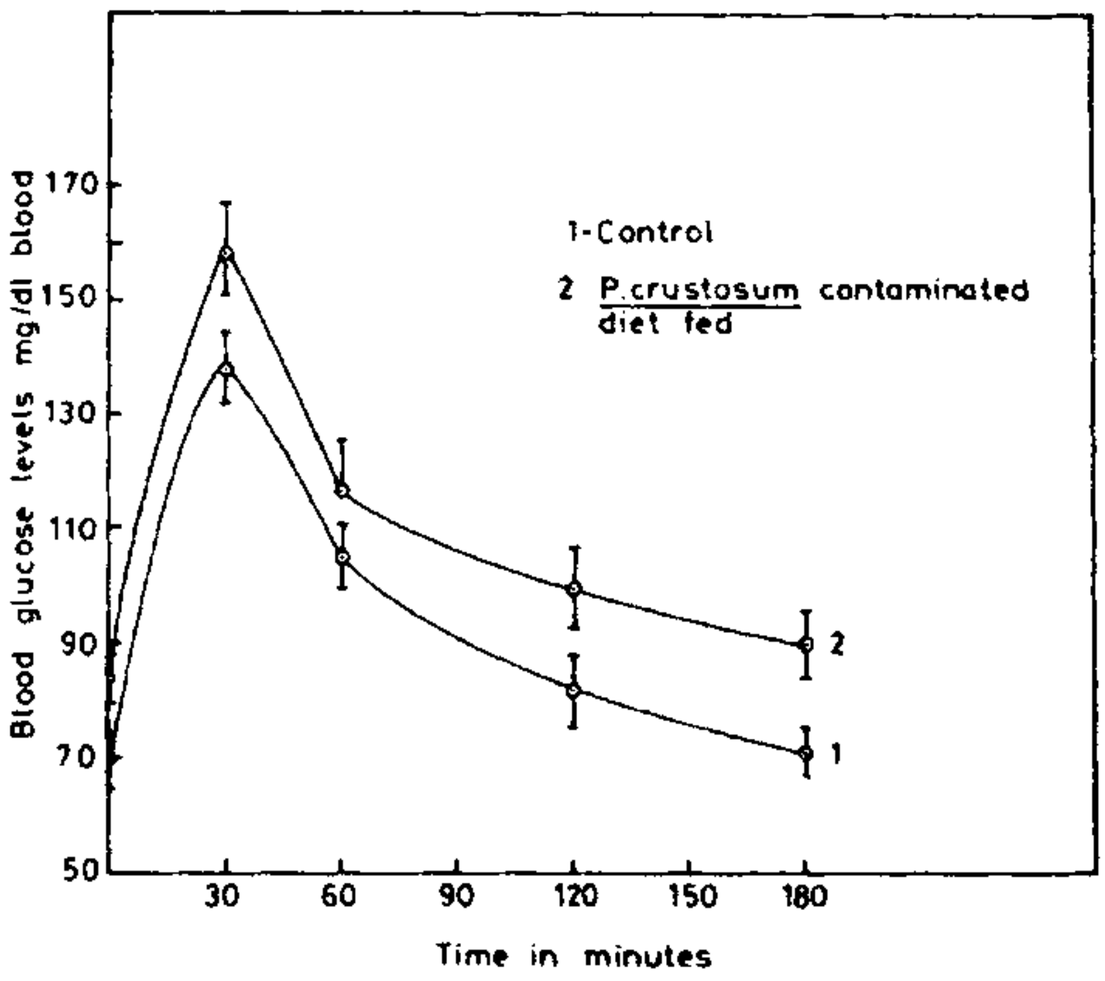

Figure 1. Glucose tolerance test conducted on control and *P. crustosum* contaminated diet fed rats.