It is suggested that when drive level is defined in terms of hours of deprivation, the animals' prior history of maintenance schedules must be taken into account. Experiments on the effects of deprivation experiences occurring in infancy are now in progress.

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References and Notes

1. J. B. Matter, Ph.D. dissertation, Radcliffe College, Cambridge, Mass., 1956. The advice of Jerome S. Bruner is gratefully acknowledged.
2. A further replication of this experiment was attempted, with 3 days allowed between blocks of five trials instead of 2 days. Under these circumstances the animals were unable to learn the task.
3. For an abstract of these studies see J. Matter, J. S. Bruner, and D. D. O'Dowd, *Am. Psychol.* 9, 427 (1954); J. S. Bruner *et al.*, *Psychol. Monographs*, in press.
4. It can be seen from the standard deviations that the distributions, especially for the latency measure, tended to be skewed. Scores were transformed for purposes of statistical analysis.

1 July 1957

Release of 5-Hydroxytryptamine by Benzoquinolizine Derivatives with Sedative Action

Previous investigations have shown that reserpine causes release of 5-hydroxytryptamine (5-HT) from various body depots (brain, intestine, and blood platelets). After a single injection of a large dose of reserpine, the 5-HT content of these organs decreased to values between one-fifth and one-tenth of the normal levels and remained low for several days. Among the *Rauwolfia* alkaloids, only those with tranquilizing action showed this effect. A series of centrally acting drugs belonging to other chemical groups did not influence the 5-HT content of the brain (*1*).

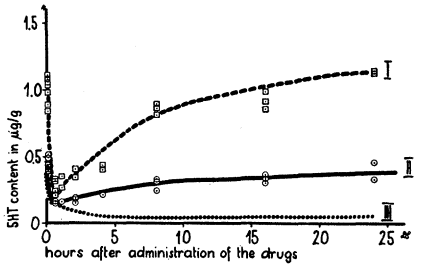
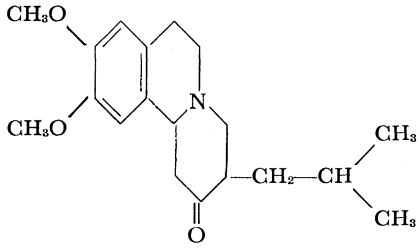


Fig. 1. Effect of compound I and reserpine on the 5-hydroxytryptamine (5-HT) content of brain. The drugs were given at zero time. Broken curve, intraperitoneal injection of 40 mg/kg of compound I to mice; each point represents the 5-HT concentration of five pooled brains. Solid curve, intravenous injection of 40 mg/kg of compound I to rabbits; each point represents the 5-HT concentration of one whole brain. Dotted curve, intravenous injection of 5 mg/kg of reserpine to rabbits (*3*).

It has now been found that, besides reserpine, various synthetic derivatives of 1,2,3,4,6,7-hexahydrobenzo[a]quinolizines (*2*) also release 5-HT. In mice and rabbits, these compounds produce sedation without hypnosis. Among the derivatives examined, compound I (2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine)



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showed the most marked sedative and 5-HT-releasing activity (Fig. 1).

After injection of 40 mg of compound I per kilogram, there was an immediate decrease of the brain 5-HT, measured fluorimetrically (*3*), the minimum value being reached within half an hour. As the dose was reduced, the 5-HT decline became gradually smaller, but was still evident with as little as 5 mg of compound I per kilogram. The absolute decrease in 5-HT per gram of tissue was greater in the brain stem than it was in the rest of the brain. During a 4-hour period after injection of 40 mg/kg in rabbits, the colorimetrically determined excretion of 5-hydroxyindoleacetic acid (*4*), a major metabolite of 5-HT, showed an average significant increase of 200 percent as compared with a similar control period before injection ($p < 0.01$). In rabbits, pretreatment with isopropyl isonicotinic acid hydrazide had the same influence on the effect of compound I as on that of reserpine (*5*): compound I no longer caused sedation, but excitation, mydriasis, and piloerection; the brain 5-HT showed only a very slight decline.

In addition to these similarities between the action of compound I and reserpine on the brain 5-HT, there were, however, some differences. (i) To reach maximum depression of 5-HT in the brain, mice required 4 times and rabbits 10 times as much compound I as was required of reserpine. In mice, the LD_{50} of compound I was about 10 times higher than the LD_{50} of reserpine. (ii) After administration of reserpine, the 5-HT in the brain decreased to a minimum of 10 percent, whereas after administration of compound I, the 5-HT concentration was never less than 25 to 35 percent of the original value. Even with doses exceeding 40 mg of compound I per kilogram, no greater decrease of 5-HT could be produced. (iii) Within 10 to 24 hours after injection of compound I, the 5-HT content of the brain had returned to normal values, whereas, after administration of reserpine, complete 5-HT recovery took several days. The sedative action of compound I lasted 4 to 8 hours, that of reserpine 1 to 3 days.

The benzo-quinolizine derivatives are thus a second group of substances which, like the centrally acting *Rauwolfia* alkaloids, cause both sedation and 5-HT depression in the brain. Closer investigations with these compounds may lead to further explanation of the role of 5-HT in brain function and in the central action of certain drugs.

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13 May 1957

Nature of the Glucuronide in Direct-Reacting Bilirubin

Evidence from several laboratories (*1–3*) has established that direct-reacting bilirubin is a diglucuronide. Billing, Cole, and Lathe (*1*) have suggested that bilirubin may be conjugated with glucuronic acid through its carboxyl groups, since the glucuronide is readily hydrolyzed by dilute alkali. Schmid (*3*) has assumed that the glucuronidic linkages occur with the α,α′-hydroxy groups of bilirubin.

Carboxyl (acyl) glucuronides can be differentiated from other glucuronides by the capacity of the former to react with hydroxylamine to yield hydroxamic acids and glucuronic acid (*4*). Treatment of bilirubin diglucuronide with hydroxylamine, therefore, provides a means of determining the nature of the glucuronidic bonds (*5*).

Urines obtained from three patients with obstructive jaundice were the source of the direct-reacting bilirubin (*3*). In each instance, treatment of an aliquot of the urine with hydroxylamine (*p*H 7, room temperature, 30 minutes) resulted in the formation of an appreciable quantity of hydroxamate. When the urine

samples were treated with 0.2*N* NaOH for 10 minutes at room temperature, the direct-reacting bilirubin was converted to indirect-reacting bilirubin, and the capacity to form hydroxamate was lost. The data presented in Table 1 show a fairly constant proportionality between the decrease in hydroxamate formation and the amount of direct-reacting bilirubin converted to the indirect-reacting form.

The direct-reacting bilirubin in urine was converted to the stable azopigment B (*3*) by coupling with diazotized sulfanilic acid. The product was extracted with *n*-butanol, purified by ascending chromatography on washed Whatman No. 3 paper, with water-saturated *n*-butanol, acetic acid (4/1) as the solvent, and eluted with absolute methanol. Azopigment B can be converted to azopigment A, with the liberation of glucuronic acid, either by dilute alkali (0.2*N* NaOH, 10 minutes, room temperature), by acid (1*N* H_2SO_4, 80 minutes, 100°C), or by the action of bacterial β-glucuronidase (Sigma, 20 mg/ml, *p*H 6, 38°C, 18 hours) (*1, 3*). With ascending chromatography on Whatman No. 1 paper and with water-saturated *n*-butanol, acetic acid (4/1) as the solvent, azo-pigments B and A migrate with R_f values of 0.36 and 0.55, respectively.

Treatment of azopigment B with an excess of hydroxylamine (*p*H 7, room temperature, 30 minutes) yields a new pigment, which migrates as a single spot with an R_f value of 0.44. This product, assumed to be the hydroxamate of azopigment A, has an absorption spectrum at *p*H 6.0 identical with that of azopigment B between 330 and 600 mμ, with maximal absorption at 525 mμ. In contrast to azopigment B, the hydroxamate is free of hexuronic acid, estimated by the carbazole method (*6*), and is unchanged by dilute alkali or bacterial β-glucuronidase, as judged by paper chromatography. However, on treatment with 1*N* H_2SO_4 for 80 minutes at 100°C, the hydroxamate is converted to a pigment with an R_f value identical to that of azopigment A.

These observations indicate that bilirubin diglucuronide, and the azopigment B derived from it, react with hydroxylamine to form the corresponding hydroxamic acids. This reaction, specific for *acyl* glucuronides, provides direct confirmation that bilirubin is conjugated with glucuronic acid through its carboxyl groups.

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Table 1. Effects of mild alkaline hydrolysis on the van den Bergh reaction and on hydroxamate formation in the urine of patients with obstructive jaundice. The van den Bergh reaction was performed according to the method of Malloy and Evelyn (*7*), and hydroxamate was estimated as has been previously described (*4*); both are expressed in units of optical density.

Patient	Δ van den Bergh		Δ Hydroxamate
	Direct	Indirect	
D	−190	+150	−084
S	−616	+464	−226
N	−920	+696	−367

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17 June 1957

Absence of Chitin in Epicuticle of Some Indian Scorpions

Epicuticle of arthropods is defined by Richards (*1*) as a characteristically nonchitinous layer. Krishnan, Ramachandran, and Santanam (*2*), on the other hand, demonstrated the presence of chitin in the epicuticle of a scorpion *Palamneus swammerdami* (*3*). It was therefore decided to test the epicuticle of other scorpions for chitin.

The scorpions *Palamnaeus bengalensis* and *Buthus tamulus gangeticus,* commonly found at Lucknow, were used for the study. When pieces of cleaned cuticle of freshly killed scorpions were subjected to the chitosan test, the epicuticle gave a negative reaction for chitin. Some pieces of cuticle which had been previously treated with alkali during the chitosan test were placed in 3 percent acetic acid overnight, but the epicuticle did not show dissolution. This would suggest further that the epicuticle is nonchitinous.

Krishnan *et al.* (*2*) had suggested that the epicuticle of *P. swammerdami,* unless previously treated with chlorated nitric acid, would not respond to the chitosan test. Even repetition of Krishnan's method in the cuticle of *P. bengalensis* and *B. tamulus gangeticus* did not give, with 1 percent sulfuric acid, any violet coloration characteristic of chitosan in the epicuticle. This would indicate that chitin is absent from the epicuticle of the scorpions *P. bengalensis* and *B. tamulus gangeticus.*

X-ray diffraction studies were also carried out in order to detect the presence, if any, of chitin in the epicuticle of these scorpions. Pieces of cuticle of the two scorpions under experiment were treated with several changes of cold, freshly prepared concentrated chlorated nitric acid till the epicuticle was completely freed of the underlying exocuticular material. Pieces of epicuticle thus obtained were thoroughly washed in running water and then in distilled water. For the x-ray diffraction experiment, 12 pieces were mounted, one over the other, on a small hole on a glass slide and left in a desiccator overnight. The pieces of epicuticle stuck together and to the slide during the drying process; after this the x-ray diffraction photographs were taken. Cu-K-alpha radiations were used, and the object was kept perpendicular to the beam and at a distance of 5 cm from the photographic plate.

Figures 1 and 2 show the diffraction patterns of the epicuticle of *P. bengalensis* and *B. tamulus gangeticus,* respectively. Prominent d-spacings in the epicuticle of *P. bengalensis* and *B. tamulus gangeticus* occur at 5.302 A and 3.901 A (faint) and at 3.007 A, 3.941 A, and 5.219 A, respectively.

Krishnan *et al.* (*2*) have suggested the presence of chitin in the epicuticle of *P. swammerdami* on a comparison of the d-spacings 3.37 A, 4.1 A, and 9.1 A obtained in the epicuticle after removal of the outer paraffin layer present in their scorpion, and of the d-spacings 2.22 A, 2.44 A, 3.7 A, and 4.14 A obtained in the entire epicuticle, with the d-spacings 3.3 A, 4.55 A, 5.0 A, and 9.9 A obtained in the purified chitin of *P. swammerdami.* The d-spacings obtained in the epicuticle of the scorpions *P. bengalensis* and *B. tamulus gangeticus* are different from all the d-spacings obtained by Krishnan in *P. swammerdami.* Furthermore, the d-spacings of the epicuticle of *P. bengalensis* and *B. tamulus gangeticus* do not tally with the d-spacings of chitin from other arthropods.

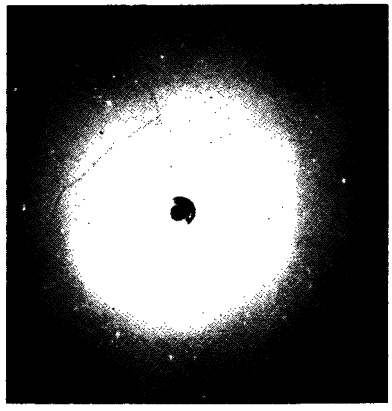

Fig. 1. X-ray diffraction photograph of the epicuticle of *P. bengalensis.*