sulted in loss of activity. The significance of the methyl group at position 1 was indicated by the reduced activity of the compound containing an ethyl group (*6*). This was also shown when the group on the ring nitrogen was a methyl (*7*) and the methyl group at position 1 was replaced by a hydrogen atom, the resulting compound (*4*) being ineffective. In comparison with the tetrahydrocarbazole compound *2*, the cyclopentano indole (*9*) and cycloheptano indole (*10*) exhibited decreased activities of 6- and 2-fold, respectively. The same structural features for maximal inhibition of ^{3}H-NA uptake have been shown for the respective compound in the 1-aminoalkyl-1, 3, 4, 9-tetrahydrothiopyrano [3, 4-b] indole series (tandamine, ED_{50}: 0.3 mg/kg, i.p.) and 1-aminoalkyl-1, 3, 4, 9-tetrahydropyrano [3, 4-b] indole series (1, 9-dimethyl-1-[2-(dimethylamino)ethyl]-1, 3, 4, 9-tetrahydropyrano [3, 4-b] indole hydrochloride, ED_{50}: 1.5 mg/kg, i.p.)[3]. Potent antagonism of reserpine-induced ptosis has been demonstrated for compound *2* with varying activities for its analogs[8]. In this regard, the potent inhibition of the NA uptake by compound *2* and varying activities of the analogs, could be the mechanism of action of these compounds.

H77/77 is a depletor of brain catecholamines and utilizes the neuronal uptake mechanism to bring about this depletion with its action on NA depletion being blocked by compounds which are known NA uptake inhibitors[11]. Thus, inhibition by compounds of the H77/77-induced depletion of NA and DA gives a measure of inhibition of neuronal NA or DA uptake. The H77/77-induced depletion of NA was blocked by compound *2*, which exhibited a potency equivalent to that of DMI (Table 2) thus indicating that compound *2* blocks NA uptake centrally in addition to that in the periphery. Neither compound *2* nor DMI[11] alone affected endogenous NA (Table 2); endogenous DA and the H77/77-induced depletion of DA were not affected by compound *2* or DMI (not shown) indicating a specificity in action.

Potentiation of the 5-HTP-induced behavioural effects has been shown to generally correlate with the ability of compounds to inhibit the brain uptake of 5-HT[3, 7, 10], provided that the compounds are not inhibitors of monoamine oxidase or releasers of 5-HT[7, 10]. Thus, activity in this model is an indication of the ability of compounds to affect 5-HT-related mechanisms. I caused a dose-related potentiation of the 5-HTP syndrome at 25 mg/kg, i.p. (+3, Table 1), 12.5 mg/kg, i.p. (+2) and 6.25 mg/kg, i.p. (+1). None of the cycloalkanoindoles examined potentiated the 5-HTP-induced activity to at least the level of I, a known blocker of 5-HT uptake[9], and thus were considered to be relatively ineffective. Compounds *4, 6 and 8 did exhibit an activity of +2*; compound *2* showed only a +1 activity. Compound *2* was thus similar to DMI since this latter drug did not exhibit appreciable activity in the present and previously reported[2–4] studies.

The present study reveals a new series of compounds structurally related to tandamine, i.e., the 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines, which inhibit the NA uptake mechanism, the most potent compound (*2*) being equivalent to DMI and lacking an appreciable ability to affect 5-HT mechanisms thus being a relatively specific NA uptake blocker. Clinically, compound *2* possessing such a profile would be expected to be of potential use in treating endogenously depressed patients, particularly those with a decreased drive[10, 11].

Effect of milk on plasma unbound-bilirubin concentration in homozygous Gunn rat sucklings

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Summary. Sucking of milk increased plasma unbound-bilirubin concentration in the homozygous Gunn's strain of jaundiced rats. Administration of skim milk did not increase unbound-bilirubin, while a mixture of fatty acids and skim milk elevated it.

Most bilirubin is bound to albumin in the plasma. The albumin-bound bilirubin cannot penetrate tissues, while the dissociated bilirubin, called 'unbound-bilirubin', may do so and thereby injure the cells[1–3]. Thus, plasma unbound-bilirubin concentration is closely correlated to the risk of kernicterus[4–7]. In the present study, effect of milk on unbound-bilirubin concentration was investigated to ascertain whether milk could displace bilirubin from albumin in the homozygous Gunn's strain of jaundiced rats.

Materials and methods. Animals. Gunn's strain of jaundiced rats (j/j; 14-day-old) were used throughout the study. Littermates diagnosed by yellow skin colour as j/j were equally allotted to experimental and control groups. Animals were maintained on laboratory diet (NMF, Oriental Yeast Co.) and tap water.

Starvation experiments. Experimental rats were isolated from their mothers and placed in an incubator at 30 °C from 5.00 p.m. till 9.00 a.m. of the next day (16 h). Physiological saline solution was administered orally at 9.00 a.m. to compensate exactly for loss of body weight during the period. Controls were left with their mothers and nursed ad libitum through the same period. Actual sucking of milk was verified by examination of the shape and weight of stomach. Blood was sampled at 10.00 a.m.

Milk administration experiments. After starvation for 16 h as described above, experimental rats were given orally 0.8 ml each of mother's milk curd solution (a 2 g wet weight of milk curds recovered from the stomach of other sucking rats was dissolved in 1 ml of 0.1 M Na_2CO_3) at 9.00 a.m. and 11.00 a.m. The controls received physiological saline instead of milk curd solution. Blood was sampled at 3.00 p.m. Human breast milk, bovine powdered milk (1 g Meiji FM-U soft curd milk dissolved in

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6.6 ml of distilled water), skim milk (1 g Snow brand skim milk dissolved in 6.6 ml of distilled water) or a mixture of skim milk and fatty acids (4.3 g skim milk, 0.24 g stearic acid, 0.24 g palmitic acid, and 0.24 g oleic acid dissolved in 33 ml of distilled water) were administered in the same way as milk curds.

Chemical analyses. The gel filtration method of Chunga and Lardinois was used for the determination of plasma unbound-bilirubin concentration[8]. Bovine serum albumin was used instead of human serum albumin for the elution of unbound-bilirubin from the column. Plasma-free fatty acid concentration was measured by the method of Lappin[9]. Plasma total protein was determined by the biuret method[10]. Albumin concentration was estimated colorimetrically after electrophoretic separation of plasma protein on a cellulose acetate membrane and stained by Ponceau 3R. Bilirubin concentration was determined according to the method of Malloy and Evelyn[11] with a slight modification for the assay of low concentration of bilirubin: in brief, an eight-fold concentrated diazo reagent was used. The reaction mixture contained 0.32 ml of plasma, 0.1 ml of diazo reagent and 0.4 ml of methanol. Optical density at 540 nm was measured 30 min after the reaction started.

Table 1. Plasma unbound- and total bilirubin concentrations in sucking and starved homozygous Gunn rat sucklings

Treatment	No. of experiments	Unbound-bilirubin (mg/dl)	Total bilirubin (mg/dl)
Sucking (control)	28	0.47 ± 0.26	8.17 ± 2.41
Starvation	28	0.10 ± 0.04 $p < 0.01$	12.44 ± 1.56 $p < 0.01$

Each value represents the mean $\pm$ S. D.

Table 2. Effect of milk administrations on plasma unbound- and total bilirubin concentrations in homozygous Gunn rat sucklings

Treatment	No. of experiments	Unbound-bilirubin (mg/dl)	Total bilirubin (mg/dl)
Saline***	12	0.10 ± 0.01	13.30 ± 1.67
Rat milk	12	0.47 ± 0.24**	10.50 ± 3.31
Human milk	13	0.34 ± 0.25*	12.87 ± 2.61
Bottled milk	10	0.60 ± 0.26**	12.65 ± 1.94
Skim milk	10	0.08 ± 0.01	12.87 ± 2.71
Skim milk and fatty acids	11	0.25 ± 0.01**	15.25 ± 2.99**

Each value represents the mean $\pm$ S. D. Significantly different from saline group, $p < 0.05$ (*), $p < 0.01$ (**). ***Among 5 littermate control groups there were no statistically significant differences in the measured values, hence, only the values of littermate controls for those given rat milk are shown.

Table 3. Plasma-free fatty acid and albumin concentrations in sucking and starved homozygous Gunn rat sucklings

	Sucking (control) (No. of experiments)	Starvation (No. of experiments)
Free fatty acids (μM)*	2524 ± 1262 (36)	622 ± 218 (36)
Albumin (μM)	360 ± 37 (10)	371 ± 31 (10)
Molar ratio of free fatty acids/albumin	7.01	1.68
Molar ratio of bilirubin/albumin	0.39	0.56

Each value represents the mean $\pm$ S. D. *Significantly different at $p < 0.01$.

Results. It is clear from table 1 that starvation induced a marked decrease of plasma unbound-bilirubin concentration and an increase of total bilirubin (unbound-bilirubin plus albumin-bound bilirubin) concentration. Administration of rat, human and bottled milk after 16 h starvation increased significantly unbound-bilirubin concentration compared with the saline group (table 2). The skim milk administered group, however, showed no increase in unbound-bilirubin concentration. There was no significant difference in total bilirubin concentration between the milk and saline groups except for that group given a mixture of skim milk and fatty acids (table 2). Administration of a mixture of skim milk and fatty acids resulted in a significant increase not only in unbound-bilirubin ($p < 0.01$), but in total bilirubin concentration ($p < 0.01$), when compared with the saline group.

The concentration of plasma-free fatty acids and albumin of the starved and sucking groups are shown in table 3. Plasma-free fatty acid level in the sucking group was four times higher than that of the starved group. There was no significant difference in albumin concentration between them.

Discussion. The results in tables 1 and 2 indicate that sucking of milk increases plasma unbound-bilirubin concentration. Table 2 also suggests that substance(s) in milk responsible for the enhancement of unbound-bilirubin concentration may be lipid in nature, since skim milk had no effect on plasma unbound-bilirubin concentration.

Johnson et al.[12] and Melichair et al.[13] have reported that administration of a large amount of fatty acids (cotton oil and olive oil) produces the decrease of plasma total bilirubin concentration in homozygous Gunn rats and human newborns, suggesting the increase of plasma unbound-bilirubin concentration.

In our experiment, plasma-free fatty acid concentration was markedly higher in sucking homozygous Gunn rats than starved ones (table 3). Wooley and Hunter[14] demonstrated in vitro that a considerable amount of bilirubin was liberated from albumin at the molar ratio of oleate/albumin above 5 when the molar ratio of bilirubin/albumin was 1. Thiessen et al.[15] have shown that at the molar ratio of bilirubin/albumin of 0.5, displacement of bilirubin molecules from their binding sites on albumin occurs when the molar ratio of free fatty acids/albumin exceeds 4. Though our data showed the molar ratio of bilirubin/albumin of 0.39, a little lower than the value discussed above, the ratio of free fatty acids/albumin in-

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creased up to 7.01 by sucking (table 3). Furthermore, the administration of fatty acids with skim milk increased plasma unbound-bilirubin concentration (table 2). It would appear that the increase of plasma-free fatty acids was one of the causes which increased plasma unbound-bilirubin concentration by sucking in homozygous Gunn rat sucklings. The increase of plasma total bilirubin concentration by starvation in table 1 suggested an increased transfer of bilirubin from tissues to plasma due to the decrease of unbound-bilirubin concentration and a subsequent equilibrium change in bilirubin distribution[4]. No significant change in plasma total bilirubin concentration, however, was observed by milk administration (table 2, except for the case of a mixture of skim milk and fatty acids) in spite of the increase of unbound-bilirubin concentration. It seems possible, therefore, to ascribe the increase of total bilirubin concentration by starvation in table 1 to a decreased rate of hepatic clearance[16]. The reason why total bilirubin concentration increased by the administration of skim milk plus fatty acids remains unexplained.

Starinsky and Shafrir[17], and Thiessen et al.[15] suggested that plasma-free fatty acids in human newborns did not seem to reach concentrations effective for increase of unbound-bilirubin concentration. The discrepancy might be caused by the difference of species, e.g., lipid composition of milk. Since plasma-free fatty acid concentration increases transiently after lipid administration[13], the inconsistency might also derive from the difference in intervals between the last sucking and blood sampling.

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Inhibition in the rat of gastric acid secretion and cyclic AMP analogs accumulation in vitro by somatostatin

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Summary. Various somatostatin (S) analogs exhibited similar degree and similar, or shorter, duration of inhibition of basal gastric acid secretion as S in the unanesthetized rat and similar, or less, inhibition of the cyclic AMP accumulation induced by prostaglandin E_2 in the rat anterior pituitary in vitro. With the analogs examined, the gastrointestinal and pituitary receptors appear to exhibit generally similar recognition specificity with the differences within the gastrointestinal activities reflecting duration of availability rather than receptor affinity.

Somatostatin* inhibits basal gastric acid secretion in the rat[2] and pentagastrin-induced gastric acid secretion in the rat[2], dog[3] and cat[4]. Somatostatin decreases fasting plasma gastrin levels in normal subjects and prevents gastrin responses to a food stimulus in patients[5]. The elevated gastrin levels of patients with pernicious anemia and Zollinger-Ellison syndrome are decreased by somatostatin[5]. Somatostatin inhibits gastric acid secretion in normal subjects and in patients with Zollinger-Ellison syndrome[5].

Somatostatin also inhibits the release of various other hormones and the accumulation of cyclic AMP, which appears to be a mediator in the hormonal release. The basal[6,7] and prostaglandin-induced increase[6-8] in the cyclic AMP accumulation in the rat anterior pituitary in vitro are antagonized by somatostatin.

The structure-activity relationships for various somatostatin analogs have been reported with regard to their abilities to inhibit basal gastric acid secretion in the rat[2] and prostaglandin-induced cyclic AMP in the rat anterior pituitary in vitro[8]. The present studies with additional analogs further define these relationships.

Materials and methods. For the determination of the basal gastric acid secretion, male albino rats (Canadian Breeding Laboratories; 160–220 g) were chronically-implanted with two gastric cannulas as previously described[9]. The animals were fasted, stomachs perfused, and the acid in the gastric perfusate determined as previously described[2].

*H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

The method employed in the determination of the accumulation of cyclic AMP in the anterior pituitary was based upon that reported previously[10,11] and was carried out as previously described[8]. After a 60 min incubation, the incubation medium was replaced by fresh buffer and glucose, and the somatostatin analog was added; after a further 2 min incubation, 20 µl vehicle or PGE_2 (1×10^{-6} *M*) was added for the incubation period of 4 min. The vehicle employed for the PGE_2 was 0.1 ml ethanol, 0.1 ml sodium carbonate (1.8 mg/ml) and 0.8 ml water. For the assay of the cyclic AMP, the cyclic AMP was extracted from the tissues with 5% trichloracetic acid and measured by the receptor-binding assay[12] utilizing

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