PHARMACOLOGICAL STUDIES OF CAERULEIN

II. THE POSSIBILITY OF MEDIATION THROUGH THE CENTRAL NERVOUS SYSTEM

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Abstract—With low doses of caerulein given intravenously or subcutaneously, vomiting could be induced. Although vomiting was not inhibited by chlorpromazine or atropine, a strong inhibition was evident when metoclopramide was administered subcutaneously at such doses as inhibit vomiting induced by oral administration of CuSO_4 . Even high doses of caerulein failed to induce vomiting in vagotomized and splanchnicotomized dogs. With intraventricular injection, no effects were observed on blood pressure, respiration or gastrointestinal motility, and vomiting was not induced. Therefore, a reflex mechanism appears to be involved in vomiting induced by caerulein. It is suggested that the actions of caerulein may not be mediated through the central nervous system.

Caerulein is a decapeptide extracted from the skin of the Australian *Hyla caerulea* by Anastasi et al. in 1967 (1). The C-terminal sequence of amino acid is the same in caerulein, pancreozymin and gastrins as shown in chemical structures. Therefore, many investigators have reported that caerulein possesses pancreozymin-like and gastrin-like pharmacological actions (2-5). However, only a few papers have reported whether or not the actions of caerulein may be partly mediated through the central nervous system.

We have reported in a previous paper (5) that the stimulation of ileum motility and gallbladder contraction by caerulein is based on peripheral action. On the other hand, Neya et al. (6) have suggested that caerulein inhibits the intestinal motility by a stimulating action on the central sympathetic area. It has been reported that physalaemin (7) ex-

tracted from the skin of the South American amphibian, *Phrynosoma macleayi*, produces a depolarizing action on spinal motoneurons (8).

In the present paper, the mechanisms of the vomiting induced by caerulein and the effect of the intraventricular injection of caerulein on gastrointestinal motility were investigated to determine the possibility of mediation through the central nervous system.

MATERIALS AND METHODS

(1) Vomiting action in normal dogs

Mongrel dogs of both sexes weighing 7–14 kg were used. As vomiting action is affected by environmental conditions as well as the gastric state, the animals were given solid food, and the experiments were performed under conditions excluding outside sounds as much as possible. In our experiments, the term vomiting was applied only to the forceful expulsion of gastrointestinal contents through the mouth, and retching was not considered vomiting.

Effects of antiemetics: Vomiting was induced by caerulein, apomorphine or CuSO_4 at such doses as always induce vomiting. One week later, the same drugs were given to the same dogs 30 min following the s.c. injection of various antiemetics.

The number of vomiting dogs, the latency periods and the mean number of occurrences of vomiting were compared in both experiments to determine the effects of various antiemetics.

Intraventricular injection: After anesthetizing dogs with pentobarbital-sodium (30 mg/kg i.v.), a steel cannula was inserted stereotaxically into the lateral ventricle at points of R: 10, T: 10 and V: 15 according to coordinates from the dog brain atlas of Lim et al. (9), and the cannula was fixed with dental cement. Seven to ten days after the implantation, drugs were dissolved in physiological saline and injected in a volume of 0.2 ml into the lateral ventricle through a cannula.

Vagotomy and splanchnicotomy: After anesthetizing dogs with pentobarbital-sodium (30 mg/kg i.v.), vagotomy was performed just above the diaphragm in the thoracic cavity and splanchnicotomy near the adrenal gland.

(2) Motility of gastrointestinal tract and gallbladder

Mongrel dogs of both sexes weighing 8–15 kg were fasted for 24 hr, and then anesthetized with pentobarbital-sodium (30 mg/kg i.v.). During laparotomy, a balloon filled with water (5 ml) was inserted about 20 cm from the end of ileum and a vinyl tube of 1 mm diameter, filled with physiological saline, was inserted into the gallbladder. Thus intraluminal pressure of the ileum and the gallbladder were recorded on a polygraph (Nihon Koden RM-85) through transducers. In the case of stomach motility, a highly flexible metallic plate (3.5 cm $\times$ 1 cm) with a strain gauge was attached to the longitudinal muscles of the corpus ventriculi. The changes in resistance caused by stomach motility were recorded on a polygraph. Carotid artery pressure was simultaneously recorded using a transducer (Nihon Koden MPU-0.5–290).

Intravenous injections were given into the brachial vein.

(3) *Stability of caerulein in cerebrospinal fluid of dogs*

Under anesthetization with pentobarbital-sodium (30 mg/kg i.v.), 0.9 ml cerebrospinal fluid was withdrawn from the cisterna magna. After adding 0.1 ml caerulein (concentration: 20 μ g/ml), the mixture was incubated at 37°C for 30 min.

The amount of caerulein in cerebrospinal fluid was calculated from potency ratio to standard caerulein solution by the bioassay method of Nakamura et al. (10). Using isolated guinea-pig gallbladders, a 4-point assay was carried out by comparing the contraction of the gallbladder to low and high doses of standard caerulein solution of known potency with those of cerebrospinal fluid caerulein.

(4) *Drugs used*

Caerulein (FI6934, Farmitalia), chlorpromazine hydrochloride (Kyowa Hakko), metoclopramide dihydrochloride hydrate (Fujisawa Yakuhin), atropine sulfate (Tanabe Seiyaku) and apomorphine (Sankyo Seiyaku). All drugs were dissolved in physiological saline.

RESULTS

(1) *Vomiting action in normal dogs*

Vomiting action induced by caerulein, apomorphine or CuSO_4 is shown in Table 1.

No significant change in behavior was produced with 0.05 μ g/kg of caerulein given i.v. Caerulein at a dose of 0.1 μ g/kg induced sedative effects immediately after the injection. Vomiting occurred in 2 out of 6 dogs within 30 sec-3 min. Dosing over 0.5 μ g/

TABLE 1. Comparison of doses of caerulein, apomorphine and CuSO_2 which produced vomiting in dogs.

		Vomiting dogs/total	mean latency (min)	mean times of vomiting
caerulein	0.05 μ g/kg i.v.	0/6	—	—
	0.1	2/6	2.3	1.5
	0.5	3/6	1.1	4.6
	1.0	10/10	1.6	2.9
	5.0	5/5	2.3	6.2
caerulein	0.5 μ g/kg s.c.	0/5	—	—
	1.0	1/6	25.0	1.0
	3.0	6/6	13.2	5.8
	5.0	3/3	18.0	2.7
apomorphine				
	2.0 μ g/kg i.v.	0/4	—	—
	5.0	1/4	1.2	1.0
	10.0	3/4	1.2	2.7
	20.0	7/7	1.1	3.7
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	50 mg/dog p.o.	1/4	7.0	1.0
	100	2/4	21.5	2.0
	150	4/4	32.0	2.3
	200	4/4	16.8	3.8

kg resulted in a heavy sedation and prostration. Recovery from sedation required 30 min-60 min. As the dose levels were increased, the number of vomiting dogs and the mean number of occurrences of vomiting were increased, while the latency periods were reduced. At a dose of 1 $\mu\text{g/kg}$, vomiting occurred in 100% of the dogs. Caerulein induced evacuation and usually diarrhea in approx. 50% of the dogs at doses from 0.1 to 1 $\mu\text{g/kg}$ and in 100% of the dogs at a dose of 5 $\mu\text{g/kg}$.

In the case of subcutaneous injection of caerulein, vomiting occurred at doses 10 times higher than the doses of the intravenous injection. Vomiting and diarrhea resulted in 1 out of 6 dogs at a dose of 1 $\mu\text{g/kg}$ and in 100% of the dogs at a dose of 3 $\mu\text{g/kg}$. Sedation followed subcutaneous injection, though not so strong as that observed in the case of intravenous injection.

Apomorphine given i.v. at doses over 5 $\mu\text{g/kg}$ induced vomiting usually accompanied with salivation and diarrhea. Vomiting occurred in 100% of the dogs at a dose of 20 $\mu\text{g/kg}$.

In the case of oral administration of CuSO_4 , vomiting occurred at doses over 50 mg/dog, in 2 out of 4 dogs at a dose of 100 mg/dog and in 100% of the dogs at a dose of 150 mg/dog.

1. Effects of antiemetics:

The effects of antiemetics on vomiting action induced by caerulein 1 $\mu\text{g/kg}$ i.v., apomorphine 20 $\mu\text{g/kg}$ i.v. or CuSO_4 200 mg/dog p.o., which proved to induce vomiting in 100% of the dogs, were examined.

TABLE 2. Effects of chlorpromazine on caerulein- and apomorphine-induced vomiting in dogs

	vomiting dogs/total	mean latency (min)	mean times of vomiting
caerulein 1.0 $\mu\text{g/kg}$ i.v.	5/5	1.3	3.6
caerulein 1.0 $\mu\text{g/kg}$ i.v. (after one week)	5/5	1.5	3.0
caerulein 1.0 $\mu\text{g/kg}$ i.v.	10/10	1.2	2.7
+chlorpromazine 1.0 mg/kg	5/5	1.4	2.4
+ 2.0 mg/kg	5/5	2.3	1.8
apomorphine 20 $\mu\text{g/kg}$ i.v.	6/6	1.2	3.2
+chlorpromazine 0.5 mg/kg	1/3	2.7	1.0
+ 1.0 mg/kg	0/3	—	—

There was little difference between the vomiting induced by caerulein 1 $\mu\text{g/kg}$ and that induced at the same dose of caerulein given one week later to the same dogs without pretreatment of antiemetics (Table 2).

Chlorpromazine: Although chlorpromazine at doses of less than 0.5 mg/kg had no significant effect on behaviour, a dose level of 1 mg/kg produced slight sedation. When the dose was further increased to 2 mg/kg, strong sedation and ataxia occurred 20 to 30 min

after the injection and lasted for 3 hr.

Chlorpromazine at a dose of 1 mg/kg did not inhibit vomiting induced by caerulein. A dose of 2 mg/kg did not inhibit vomiting induced by caerulein at all, but slightly prolonged the latency periods (Table 2). Chlorpromazine did not influence the cathartic effect of caerulein. On the other hand, chlorpromazine at a dose of 1 mg/kg completely inhibited vomiting induced by apomorphine (Table 2).

Metoclopramide: Metoclopramide at doses between 0.05 and 1 mg/kg had no significant effect on behaviour. The vomiting induced by caerulein was slightly inhibited at a dose of 0.5 mg/kg of metoclopramide and completely at a dose of 1 mg/kg (Table 3). Metoclopramide did not influence the cathartic effect of caerulein. Vomiting induced by apomorphine was completely inhibited at a dose of 0.2 mg/kg of metoclopramide (Table 3). In regard to vomiting induced by CuSO_4 , the latency periods were prolonged at a dose of 0.5 mg/kg of metoclopramide, and vomiting was induced in only 1 out of 3 dogs at a dose of 1 mg/kg (Table 3).

TABLE 3. Effects of metoclopramide on caerulein-, apomorphine- and CuSO_4 -induced vomiting in dogs

	vomiting dogs/ total	mean latency (min)	mean times of vomiting
caerulein 1.0 $\mu\text{g/kg}$ i.v.	10/10	1.6	2.6
+ metoclopramide 0.5 mg/kg	4/5	2.2	1.3
+ 1.0 mg/kg	0/5	—	—
apomorphine 20 $\mu\text{g/kg}$ i.v.	9/9	1.1	4.3
+ metoclopramide 0.05 mg/kg	2/3	1.4	1.5
+ 0.1	1/3	2.0	3.0
+ 0.2	0/3	—	—
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 200 mg/dog p.o.	5/5	9.8	4.2
+ metoclopramide 0.5 mg/kg	2/2	26.0	4.0
+ 1.0 mg/kg	1/3	11.0	4.0

TABLE 4. Effects of atropine on caerulein-induced vomiting in dogs

	vomiting dogs/ total	mean latency (min)	mean times of vomiting
caerulein 1.0 $\mu\text{g/kg}$ i.v.	10/10	1.6	2.9
+ atropine 0.2 mg/kg	5/5	2.8	2.2
+ 0.5 mg/kg	5/5	1.6	2.8

Atropine: Although atropine at a dose of 0.2 mg/kg had no significant effect on behaviour, a dose level of 0.5 mg/kg alone induced restlessness and vomiting in most dogs.

Atropine at a dose of 0.2 mg/kg did not inhibit vomiting induced by caerulein, but slightly prolonged the latency periods. Atropine at a dose of 0.5 mg/kg never inhibited vomiting and never changed the latency periods or the mean number of occurrences of

vomiting (Table 4). On the contrary, atropine inhibited the cathartic effect of caerulein. After 0.5 mg/kg of atropine, diarrhea by caerulein occurred in only 1 out of 5 dogs.

2. Intraventricular injection (Table 5)

Intraventricular injection of 0.1 $\mu\text{g/kg}$ of caerulein had no significant effect on behaviour. Although licking and restlessness occurred in only 1 out of 6 dogs, these slight changes in behaviour were occasionally noted after the intraventricular injection of physiological saline. Intraventricular injection of 0.05 $\mu\text{g/kg}$ of apomorphine induced licking and deep respiration but no vomiting. Vomiting occurred in 2 out of 4 dogs at a dose of 0.1 $\mu\text{g/kg}$ and in 2 out of 3 dogs at a dose of 2 $\mu\text{g/kg}$.

TABLE 5. Vomiting induced by intraventricular injection of caerulein and apomorphine.

		vomiting dogs/ total	mean latency (min)	mean times of vomiting
caerulein	0.1 $\mu\text{g/kg}$	0/6	—	—
apomorphine	0.05 $\mu\text{g/kg}$	0/4	—	—
	0.1	2/4	2.5	2.5
	2.0	2/3	4.2	3.0

TABLE 6. Vomiting induced by caerulein, apomorphine and CuSO_4 in normal and vagotonized & splanchnicotomized dogs.

		vomiting dogs/ total	mean latency (min)	mean times of vomiting
Normal	caerulein 1.0 $\mu\text{g/kg}$ i.v.	10/10	1.6	2.9
	5.0	5/5	2.3	6.2
	apomorphine 20 $\mu\text{g/kg}$ i.v.	7/7	1.1	3.7
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 200 mg/dog p.o.	4/4	16.8	3.8
vagotonized & splanchnicotomized	caerulein 1.0 $\mu\text{g/kg}$ i.v.	0/5	—	—
	5.0	0/5	—	—
	apomorphine 20 $\mu\text{g/kg}$ i.v.	3/3	1.5	4.3
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 200 mg/dog p.o.	0/5	—	—
vagotonized	caerulein 1.0 $\mu\text{g/kg}$ i.v.	1/5	8.5	2.0
	5.0	3/5	1.9	5.7
splanchnicotomized	caerulein 1.0 $\mu\text{g/kg}$ i.v.	4/5	1.6	3.8
	5.0	5/5	1.3	5.4

3. Vagotomy and splanchnicotomy (Table 6)

Vomiting occurred in all the vagotonized and splanchnicotomized dogs by the i.v. injection of 20 $\mu\text{g/kg}$ of apomorphine. The latency periods and the mean number of occurrences of vomiting were almost identical with those in normal dogs. Vomiting did not occur in the vagotonized and splanchnicotomized dogs by the i.v. injections of 1 $\mu\text{g/kg}$ and 5 $\mu\text{g/kg}$ of caerulein, but diarrhea occurred in approx. 50% of the dogs. Vomiting did not occur when an oral administration of 200 mg/dog of CuSO_4 was given.

Vomiting occurred in only 1 out of 5 vagotomized dogs at a dose of $1 \mu\text{g}/\text{kg}$ of caerulein and in 3 out of 5 vagotomized dogs at a dose of $5 \mu\text{g}/\text{kg}$. On the other hand, an increase in the threshold dose of caerulein inducing vomiting was noted rarely after splanchnicotomy alone.

(2) *Effects of caerulein on the motility of gastrointestinal tract and gallbladder*

Caerulein stimulated the motility of the stomach and the ileum. The i.v. threshold doses were $1\text{--}5 \text{ ng}/\text{kg}$ in the stomach and $5\text{--}10 \text{ ng}/\text{kg}$ in the ileum. An increase in rhythmic amplitude was induced immediately after the injection and an increase in tonus as well was induced at high doses. Tachyphylaxis was lacking.

Caerulein caused a constant contraction of the gallbladder after a short latency period, at very low doses. In most experiments, the i.v. threshold doses ranged between 0.3 and $0.5 \text{ ng}/\text{kg}$. Tachyphylaxis or sensitization was not present. A good dose-response relationship was observed up to approx. $100 \text{ ng}/\text{kg}$.

While the contraction of the gallbladder by $30 \text{ ng}/\text{kg}$ of caerulein was not affected at all by atropine $0.2 \text{ mg}/\text{kg}$ i.v., the stimulation of the motility of the stomach and the ileum was strongly inhibited ($50\text{--}70\%$) (Fig. 1). The same dose of atropine completely inhibited the action of acetylcholine.

FIG. 1. Effects of atropine on caerulein-induced gallbladder contraction and gastrointestinal motility.

B.P.; Blood pressure, G.S.; Gallbladder, S; Stomach motility, I; Ileum motility, caer.; caerulein

FIG. 2. Dose-response curve and time-course for the lowering of blood pressure after caerulein given intravenously. caer.; caerulein Mean $\pm$ S.E. $N=6$

Caerulein produced hypotension immediately after the i.v. injection. The threshold doses ranged between 30 and 50 ng/kg. Dose-response relationship for the lowering of mean arterial blood pressure by caerulein was observed. Duration of hypotension, even at high doses, was approx. 30 min (Fig. 2).

The stimulation of gastrointestinal motility was induced by the i.v. injection of 10 ng/kg of caerulein. The lowering of blood pressure, the increase in respiratory rate and the continuous stimulation of gastrointestinal motility were induced at a dose of 30 ng/kg immediately after this i.v. injection, but never with the intraventricular injections of 1 ng/kg and 10 ng/kg of caerulein (Fig. 3).

FIG. 3. Effects of the intraventricular injection of caerulein on blood pressure, respiration and gastrointestinal motility.

B.P.; Blood pressure, S; Stomach motility, I; Ileum motility, caer.; caerulein

(3) Stability of caerulein in cerebrospinal fluid of dogs

The potency of caerulein was not degraded at all in cerebrospinal fluid. The potency ratio of caerulein in cerebrospinal fluid to the standard caerulein solution (=100) was 97.0 (95% confidence limit: 73.5–127.9).

DISCUSSION

It is known that the dog among all the species of animals is the most sensitive to vomiting (11). Sensitivity to caerulein is high in dogs, and vomiting occurred at 0.1 $\mu\text{g/kg}$ i.v. and 1 $\mu\text{g/kg}$ s.c., in accordance with the report by Bertaccini et al. (2).

However, the dose of caerulein inducing vomiting was 10–500 times higher than the threshold doses of caerulein capable of producing gallbladder contraction, stimulation of gastrointestinal motility and pancreatic secretion (4). This indicates that caerulein is a drug with a wide margin of safety in clinical usage.

It is established that chlorpromazine blocks the medullary emetic chemoreceptor

trigger zone. Although chlorpromazine inhibited central vomiting by apomorphine as previously reported (12), chlorpromazine at doses not inducing sedation or ataxia did not inhibit caerulein-induced vomiting at all. Laville (13) has reported that metoclopramide inhibits reflex vomiting as well as central one. Metoclopramide inhibited caerulein-induced vomiting at such doses as inhibit reflex vomiting by CuSO_4 given p.o. The intraventricular injection of apomorphine induced vomiting at low doses as previously reported (14). But the intraventricular injection of caerulein did not induce vomiting at all. Apomorphine induced vomiting in the vagotomized and splanchnicotomized dogs as well as in the normal dogs. Neither caerulein nor CuSO_4 induced vomiting even at high doses in the vagotomized and splanchnicotomized dogs. From the above results, caerulein-induced vomiting appears to be reflex vomiting induced by impulses being transmitted centrally via afferent fibers.

An increase in the threshold dose of caerulein inducing vomiting was noted after vagotomy alone, whereas change in the threshold dose was noted rarely after splanchnicotomy. This is in accordance with the results of CuSO_4 - and enterotoxin-induced vomiting (15-16). Therefore, it is suggested that the main nervous pathway in caerulein-induced vomiting is the vagus.

Bertaccini et al. (2) have reported in a preliminary experiment that atropine retards caerulein-induced vomiting. In our experiment, although slight prolongation of the latency periods was caused by atropine at a dose not inducing vomiting by itself, occurrence of vomiting was not at all inhibited.

The stimulation of gastrointestinal motility and gastric secretion (3) among peripheral actions of caerulein were inhibited by atropine, whereas gallbladder contraction, pyloric spasm (2), (17) and pancreatic secretion (4) were not at all affected.

From the results of the effects of atropine, it is probable that pyloric spasm, pancreatic secretion and gallbladder contraction are concerned with vomiting. The fact that the pyloric spasm (18) has been found to induce vomiting suggests that pyloric spasm, in particular, plays important roles in caerulein-induced vomiting.

Caerulein induced diarrhea in the vagotomized and splanchnicotomized dogs as well as in normal ones. The cathartic action of caerulein was inhibited by atropine. These results suggest that the cathartic actions appears to be induced by stimulation of gastrointestinal motility.

In regard to central action of caerulein, Neya et al. (6) have reported that caerulein probably possesses a stimulating action on the central sympathetic area, from the result that the action of caerulein on jejunal motility was potentiated after the inhibition of sympathetic action by procedures such as bilateral splanchnicotomy, transection of spinal cord or decerebration.

Paton and Vizi (19) have reported that acetylcholine release is under normal control of the sympathetic, therefore, withdrawal of sympathetic control will lead to the potentiation of parasympathetic response. In addition, Jacobowitz (20) and Norberg and Sjöqvist (21) have demonstrated this anatomically.

It has already been confirmed that caerulein stimulates gastrointestinal motility by releasing acetylcholine from cholinergic nerve endings (2, 5).

From above mentioned findings, it is reasonable to speculate that the potentiation of the action of caerulein after bilateral splanchnicotomy is due to acetylcholine release having been accelerated rapidly by the withdrawal of sympathetic control. Therefore, it is unlikely that caerulein possesses a stimulating action on the central sympathetic area. The following results in this experiment appear to support the hypothesis that the actions of caerulein are not mediated through the central nervous system. a) Caerulein-induced vomiting appears to be reflex vomiting. b) Caerulein induced vomiting and strong hypotension at such doses as induced sedation. Durations of these effects were almost identical. Therefore, the sedation by caerulein appears to be accompanied with nausea and hypotension. c) Intraventricular injection of caerulein did not affect blood pressure, respiration or gastrointestinal motility. This result would not be due to degradation of caerulein by cerebrospinal fluid.

REFERENCES

- 1) ANASTASI, A., ERSPAMER, V. AND ENDEAN, R.: *Experientia* **23**, 699 (1967)
- 2) BERTACCINI, G., DE CARO, G., ENDEAN, R., ERSPAMER, V. AND IMPICCIATORE, M.: *Brit. J. Pharmacol.* **34**, 291 (1968)
- 3) BERTACCINI, G., ENDEAN, R., ERSPAMER, V. AND IMPICCIATORE, M.: *Brit. J. Pharmacol.* **34**, 311 (1968)
- 4) BERTACCINI, G., DE CARO, G., ENDEAN, R., ERSPAMER, V. AND IMPICCIATORE, M.: *Brit. J. Pharmacol.* **37**, 185 (1969)
- 5) NAKAMURA, N., KOYAMA, Y., KOJIMA, T. AND TAKAHIRA, H.: *Japan. J. Pharmacol.* **23**, 107 (1973)
- 6) NEYA, T., TSUCHIYA, K., WATANABE, K., TAKEDA, M. AND YAMASATO, T.: *Japan., J. Smooth Muscle Res.* **9**, 63 (1973)
- 7) BERTACCINI, G., CHU, J.M. AND ERSPAMER, V.: *Brit. J. Pharmacol.* **25**, 363 (1965)
- 8) KONISHI, S. AND OTSUKA, M.: *Japan., J. Pharmacol.* **21**, 682 (1971)
- 9) LIM, B.K.S., LIU, C.N. AND MOFFITT, R.L.: *A Stereotaxic Atlas of the Dog's Brain*, Published by CHARLES C. THOMAS (1960)
- 10) NAKAMURA, N., KOYAMA, Y., TANAKA, Y., KOJIMA, T. AND MARUMO, H.: *Pharmacometrics.* **8**, 865 (1974)
- 11) LAFFAN, R.L. AND BORISON, H.L.: *J. Pharmacol. exp. Ther.* **121**, 468 (1957)
- 12) BRAND, E.D., HARRIS, T.D., BORISON, H.L. AND GOODMAN, L.S.: *J. Pharmacol. exp. Ther.* **110**, 86 (1954)
- 13) LAVILLE, C.: *Path. Biol.* **12**, 577 (1964)
- 14) SHIARE, N.N., CHAI, C.Y. AND WANG, S.C.: *J. Pharmacol. exp. Ther.* **147**, 416 (1965)
- 15) WANG, S.C. AND BORISON, H.L.: *Am. J. Physiol.* **164**, 520 (1951)
- 16) CLARK, W.G., VANDERHOOF, G.F. AND BORISON, H.L.: *Proc. Soc. exp. Biol. Med.* **111**, 205 (1962)
- 17) MANTOVANI, P. AND BERTACCINI, G.: *Arch. int. Pharmacodyn. Ther.* **193**, 362 (1971)
- 18) NETTER, F.H.: *The Ciba collection of medical illustrations, Digestive System*, Vol. 3, Part 1, 89 (1971)
- 19) PATON, W.D.M. AND VIZI, E.S.: *Brit. J. Pharmacol.* **35**, 10 (1969)
- 20) JACOBOWITZ, D.: *J. Pharmacol. exp. Ther.* **149**, 358 (1965)
- 21) NORBERG, K.A. AND SJÖQVIST, F.: *Pharmacol. Rev.* **18**, 743 (1966)