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Review Article

Distribution and Biological Effects of Substance P

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CONTENTS

EXTENSIVE INVESTIGATIONS in recent years of the physiological and pharmacological properties of endogenous biologically active substances have increased interest in the polypeptide substance P (SP). Although SP is one of the oldest known peptides, discovered by Euler and Gaddum (1) in 1931 while investigating acetylcholine distribution, its biological function is largely unknown. Many speculative functions have been proposed for SP; as an essential factor for the rhythmic motility of the intestine (2–5), both as an excitatory and inhibitory neurotransmitter (6–9), as a general hyperpolarizing modulator (10, 11) or physiological tranquilizer (12, 13), as a carrier of various transmitter substances (14–16), and as a capillary permeability increasing agent (17, 18), but conclusive evidence for any or several of these functions has not been obtained.

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Substance P exerts its pharmacological effects primarily on three organic systems: the central nervous, gastrointestinal, and circulatory systems. While the initial publication described SP as a smooth muscle stimulating substance and blood pressure depressor, it also affects the central nervous system. This review will summarize the knowledge of SP to the present, particularly on these three systems. Other reviews (19–27) cover additional details and aspects.

CHEMICAL CHARACTERISTICS

Substance P is a basic straight chain polypeptide, with molecular weight 1650 ± 350 (19, 28). Total hydrolysis gives the following 13 amino acids: lysine, arginine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, leucine, isoleucine, phenylalanine, threonine, and serine (28–31). Arginine-proline-proline are the first three amino acids from the *N*-terminus, as in bradykinin, but the remainder of the chain is different (29, 32). Owing to the extreme instability of purified SP in solution, the specific sequence and synthesis have not been determined. A 10^{-6} dilution of the purified substance loses over half of its activity within 2 min., while a 10^{-3} dilution is stable for several hours (28). Cleugh and Gaddum (33) suggest that this instability of the pure SP is due to its adsorption properties.

Substance P is very soluble in water, methanol, ethanol, and acetic acid and is insoluble in ether and chloroform. It can be precipitated from

alcoholic solutions with acetone or picric acid, and from watery solutions with phosphotungstic acid, mercuric chloride, or by 70% saturation with ammonium sulfate. Substance P is thermostable from pH 1–7, but activity is rapidly lost in an alkaline state. It dialyzes rapidly through cellophane, parchment, and collodion membranes (2, 34).

Paper chromatography using different solvent systems: *n*-butanol–acetic acid–water (40:10:50), *n*-butanol–pyridine–acetic acid–water (30:20:6:24), and pyridine–acetic acid–water (30:50:15) gave R_f values for SP of 0.34–0.37, 0.46, 0.61, respectively (21, 31, 35). Zetler (35) found that SP migrated 116 mm. to the cathode during paper electrophoresis (pH 4.95, acetate buffer, duration 6 hr.). SP migrates to the cathode at pH below 10.5. Using the buffer system formic acid–acetic acid–water (15:10:75) and pH 1.9, bovine SP migrated at a velocity 0.8 times that of glutamic acid (28); horse SP, however, migrated 1.0 times that of glutamic acid (29). The isoelectric point was 8.6 ± 0.2 (28). In countercurrent distribution in the system *n*-butanol–pyridine–acetic acid–water (40:10:5:45) at 20°, SP gave a value of K = 0.61 (28).

No specific chemical reaction for substance P is known, so SP concentration is estimated using bioassays, comparing the response against a standard preparation. One unit of SP is contained in approximately 20–30 mg. of horse intestine (34). Purified SP contains 120,000–150,000 units of activity per milligram (unit/mg.) (28, 30).

The isolated guinea pig ileum provides the most specific assay available (36). The most commonly used assay (37) uses a segment from the distal ileum in a 2–5-ml. intestinal bath containing Tyrode's solution at 34–38°. Hyoscine (10^{-8} Gm./ml.), pyrilamine[1] (10^{-6} Gm./ml.), UML 491 (5×10^{-8} Gm./ml.), or other suitable blocking agents, which will antagonize acetylcholine, histamine, and serotonin, respectively, are added to the bath solution. Many other active substances which may be present in crude extracts can be eliminated by specific tachyphylaxis (36) or by enzymatic deamination (38, 39). The contraction curve for SP is very similar to acetylcholine, with the exceptions of a smaller maximum and a slightly delayed onset and maximum response time (28). Maximum contraction occurs usually within 1 min. and doses can be repeated at 3–4-min. intervals (37). In this bath 0.2 unit/ml. should give a good response and a 10% change can be detected. In a 0.05-ml. microbath the guinea pig ileum is sensitive to 0.001–0.01 unit of SP (40). Purified SP (50,000 units/mg.) in a dose of 10^{-9} Gm./ml. caused a submaximal contraction of the isolated guinea pig ileum, so SP on a molar basis is about 10 times more active than acetylcholine (28). This sample produced no tachyphylactic phenomenon, even with the time interval as short as 1 min., which is opposite to the results obtained using impure SP (41). Substance P stimulates the longitudinal but not the circular muscle (42). Below 20° contractions are inhibited (43) and highly variable results occur after storage (43, 44). Substance P is reported to increase in potency with increased pH (45) or to be unaffected (46).

Only two reasonably specific SP antagonists have been found: cystine-di-β-naphthylamide and trimethaphane-*d*-camphorsulfonate.[2] They have a slight antagonist effect against acetylcholine, histamine, and 5-hydroxytryptamine, but no effect on oxytocin, vasopressin, and bradykinin (47, 48).

The contraction of the isolated hen rectal caecum, rabbit jejunum, goldfish intestine, and fall in blood pressure of the atropinized rabbit are other bioassays which are sensitive to purified SP as well as crude preparations (36, 49–54).

Three extraction procedures are in common use. The standard extraction procedure consists of boiling the tissue at pH 4, precipitation of inactive protein by adding ethanol, and further concentrating SP by precipitation with ammonium sulfate (37, 55). Substance P is extracted from small quantities of tissue by an acid extraction of the acetone insoluble residue (37, 56, 57). Lembeck *et al.* (58) have recently developed a new chloroform–methanol extraction procedure. Crude extracts can be further purified by adsorption on an aluminum oxide column; SP is eluted with decreasing concentrations of methanol (37). Purified SP has been obtained by elaborate procedures involving repeated adsorption and ion exchange chromatography and countercurrent distribution (28–31).

Zetler (35, 59–64) in purifying SP extracts used carbon tetrachloride to remove salts and ammonium sulfate from the extract. Substance P from cattle brain treated in this way yields three biologically active polypeptides, while extracts from the horse intestinal tract gave ten. While some of the peptides may be artifacts from protein denaturation, they are probably

[1] Marketed as Neo-antergan by Merck & Co., Inc., Rahway, N. J.

[2] Marketed as Arfonad by Roche Laboratories, Nutley, N. J.

not all so. One of the active peptides was found in both the brain and intestine, which also had all of the properties of SP (62, 65). Zetler (62) concluded that the brain F_b and intestinal F_{b3} fractions are SP, which creates a slight problem, as Lembeck *et al.* (58) in their new extraction method, extract only fraction F_a from nervous tissue and found no SP activity remaining in the tissue after extraction.

Biological activity of highly purified SP is completely destroyed by chymotrypsin, but only partially by trypsin (32, 54). Impure SP is also inactivated by pepsin, cathepsin, papain, diamine oxidase, bacterial proteolytic enzymes and extracts from nervous tissue, intestinal tract, urinary bladder, ureter, and uterus (66–72). Eber and Lembeck (66) found kidney and spleen extracts most potent in inactivating SP, with no relationship between the tissue SP content and its enzymatic activity. Blood serum from gravid animals destroys SP, while normal serum has no or only slight inactivation ability (73, 74). In subcellular studies an enzyme which inactivates SP appears to be located in the cytoplasm–the supernatant fraction (75, 76). Substance P is very resistant to autolysis (66, 77, 78).

DISTRIBUTION

The gastrointestinal tract and nervous system of all investigated vertebrates contain SP (21, 79–86), but none of the noncordates (87, 88). The tunicate, *Ciona intestinalis*, is the most elementary species in which SP (0.02 unit/Gm.) has been found (87). Substance P distribution in five species is shown in Table I.

In the intestinal tract, the content is low in the esophagus and stomach, high in the duodenum and jejunum. The ileum, colon, and rectum contain moderate amounts (21). The amount of SP in the intestine varies with the species, in decreasing order: dog, monkey, horse, bovine, man, rat, guinea pig, sheep, cat, pig, ray, and dogfish (21, 87, 92). While SP is found in all layers of the gastric and intestinal walls, the muscularis mucosa contains the greatest concentration (21, 96).

Amphibians have the highest total brain concentration (200–820 units/Gm.) with moderate amounts in reptiles and smaller amounts in birds, mammals, and fish (79–81). Differences in forebrain and brain stem concentrations are less in primitive than more developed brains. With increased differentiation of the central nervous system, the concentration decreases in the whole brain (81) and in specific nuclei (90). The topographic distribution shows that while SP is present in all parts, the concentration varies over a wide range. Cell-rich phylogenetic older parts of the brain have the primary concentration with the cerebellum a notable exception.

In the cortex there is an uneven distribution, the anterior cingulate gyrus has a high concentration; the olfactory, somatomotor, and somatosensory cortices have moderate amounts, and the visual cortex has little. The cerebellar cortex contains small amounts of SP, but considered as a whole there is no more than in white matter.

Closely neighboring brain stem structures show neurohumoral differentiation (78), as the thalamus (12 units/Gm.), globus pallidus (112 units/Gm.), and putamen (64 units/Gm.); anterior (70 units/Gm.) and posterior (22 units/Gm.), hypothalamus, and mammillary body (34 units/Gm.); ala cinerea (248 units/Gm.), trigonum hypoglossi (37 units/Gm.), and area postrema (290 units/Gm.); substantia nigra (699 units/Gm.) and red nucleus (30 units/Gm.).

The concentration of SP in functional systems shows significant differences; for example, the visual system, other than the retina, has much lower concentrations than the auditory system.

In the spinal cord as in the brain, the gray matter shows a higher concentration than the white. There is more SP in the dorsal than the ventral halves of the spinal cord, except in the frog where they are the same (98). This high concentration of SP in the sensory pathway: the dorsal roots, fasciculi gracilis and cuneatus, and associated nuclei with very low concentrations of other well-known pharmacologically active substances, led to Lembeck's suggestion (6) that SP may be the transmitter of the sensory neurons.

All peripheral nerves contain SP, but in much smaller concentrations than in the central nervous system. The distribution indicates the autonomic nerves, in particular the sympathetic preganglionic, contain the largest amounts (21).

Differential and density gradient centrifugation studies of brain homogenates show that the intracellular distribution of SP is similar to acetylcholine (36, 76, 99–105). The distribution is bimodal, between the supernatant ($^1/_3$) and synaptic vesicles and the incompletely disrupted synaptosomes fractions ($^2/_3$). Substance P is found, as is acetylcholine, in the hollow or type 1 synaptic vesicle. Substance P in the vesicle-synaptosomes fractions is in a bound form and must be released before assay. Lembeck *et al.* (58) think it is bound to phosphatides. Substance P is released rapidly upon exposure to hypotonic solutions or extraction at pH 4.0.

TABLE I.—REGIONAL DISTRIBUTION OF SUBSTANCE P IN FIVE SPECIES (UNITS OF ACTIVITY/GM. OF TISSUE)

Species Ref.[a]	Human (18, 78, 89–92)	Bovine (21, 78, 90, 93–95)	Dog (21, 56, 90, 94, 96, 97)	Cat (21, 81, 95)	Ray (82, 83, 87)
G.I. Tract					
Esophagus	...	2.0	Low	1.3	...
Stomach	...	7.5	Moderate	2.5	...
Duodenum	...	15.0	64.9	8.0	3.0
Jejunum	19.0	22.5	53.6	6.0	
Ileum	...	...	...	...	
oral	...	16.2	40.1	4.0	...
middle	...	13.8	36.4	2.8	...
coecal	...	10.3	39.7	3.3	...
Colon	...	...	41.3	...	...
oral	8.4	15.3	...	5.3	...
rectal	9.4	15.8	...	5.0	...
Rectum	...	16.3	28.3	5.5	...
Nervous System					
Telencephalon	...	...	...	21	5
Pallium	34	...	...	...	...
precentral gyrus	43	7	21	...	...
postcentral gyrus	39	6	20	...	...
striate cortex	...	4	14	...	...
anterior cingulate	85	...	...	...	...
lateral olfactory gyrus	...	...	29	...	...
Corpus callosum	2	...	7	...	...
Basal nuclei	102	...	128	155	...
Caudate	85	80	46	...	...
Lenticular	...	45	...	...	...
putamen	64	...	...	...	...
globus pallidus	112	...	...	...	...
Hippocampus	...	25	15	...	...
Olfactory bulb	...	...	8	...	...

(continued on next page.)

The observed intracellular distribution is similar by using various methods of preparation, or different species (rat, sheep, guinea pig, rabbit) or region of the CNS (cortex, midbrain, cerebellum, and ventral and dorsal halves of the spinal cord) except that the total amount reflects the gross regional distribution (101, 104).

Euler (76, 106) found that the intracellular distribution of SP in peripheral nerves (vagus and splenic nerves of the cow and sciatic and brachial nerves of the dog) was primarily in granules as is the case in the central nervous system.

The subcellular and regional distribution indicates that SP is found within nerve cells rather than glial cells. This is supported also by Grabner and Lembeck's finding (107) that glial tumors contain no substance P activity, and that SP increases in the proximal and decreases in the distal part of cut peripheral nerves in degeneration experiments (98, 108–110). No change occurs in SP concentration in the spinal cord of the rat after dissection between the first and second lumbar vertebrae (111).

Substance P occurs in other organs besides the nervous system and the gastrointestinal tract, but only in minute amounts (1, 21). The ureter and uterus (5 units/Gm. of tissue in the dog) show the relatively highest amounts (21). Substance P is also found in minute amounts in blood (1) but not in urine (112).

ENDOGENOUS CHANGES IN CONCENTRATION

Besides the distribution, which indicates SP's physiological role is connected with function rather than structure, there are endogenous changes under different functional states. In an ontogenetic study on the bovine fetus (113), a brain weighing only 1 Gm. has a high content (21.5 units/Gm.) of SP. The brain stem, which is active during the development of the embryo, contains SP concentrations comparable to the adult, while the forebrain has none until after birth. The intestine contains increasing amounts of SP during the second half of embryonic development. Immature undifferentiated nervous tissues, neural tumors, do not contain SP (107).

In the gravid rat, there is an increase in SP content in the uterus and a marked decrease in the brain, particularly in the second half of pregnancy (74). Up to 4 times the concentration of SP is found in the retina of cattle, whose

TABLE I.—(*Continued.*)

Species Ref.[a]	Human (18, 78, 89–92)	Bovine (21, 78, 90, 93–95)	Dog (21, 56, 90, 94, 96, 97)	Cat (21, 81, 95)	Ray (82, 83, 87)
Diencephalon	...	...	...	...	
Thalamus	12	21	13	22	
lateral geniculate	5	...	...	...	
medial geniculate	37	...	...	...	
Red nucleus	30	...	...	...	
Substantia nigra	710	...	...	...	8
Hypothalamus	102	83	70	...	
mammillary bodies	34	...	...	...	
Mesencephalon	...	...	68	...	
Corpora quadrigemia	...	...	20	...	...
superior colliculus	55	92	...	55	...
inferior colliculus	141	122	...	44	...
Tegmentum	...	108	74	...	...
Cerebral peduncles	...	45	41	...	...
Substantia grisea centralis	119	149	...	...	...
Cerebellum	2	1	2	4	...
Medulla oblongata	...	...	35	...	39
Pons	3	...	21	52	...
Floor of fourth ventricle	...	34	45	...	...
ala cinerea	248	410	...	...	...
trigonum hypoglossi	37	34	...	...	...
area postrema	...	143	375	...	...
Spinal cord	...	...	34	...	23
Grey matter	...	...	68	...	...
White matter	...	...	9	...	...
Dorsal half	...	166	27	...	25
Ventral half	...	47	6	...	15
Nucleus gracilis	49	165	79	...	...
Nucleus cuneatus	25	125	55	...	...
Other tissue	...	...	...	...	...
Retina	...	5	24	...	...
Optic nerve	...	16	6	...	...
Dorsal roots	...	25	40	...	...
Ventral roots	...	3	6	...	...
Vagus nerve	...	5	22	...	...

[a] Kopera and Lazarini (95) and Douglas *et al.* (96) used different standards from the other authors to measure the biological activity.

eyes were closed for 2 hr. as compared to those illuminated for the same length of time. The extent of change from normal is about the same for each case. No change occurs in the SP content of an isolated retina *in vitro* to illumination or darkness (114). However, in the brain of the rat and the rabbit the content increases after illumination and decreases after darkness or blindness (115, 116). Stern (116) measured also changes in brain concentration of the rat when deprived of other sensory stimuli. Elimination of smell and hearing reduced SP content, while elimination of the tactile sense or the "radar apparatus" in the bat had the opposite effect. Vestibular stimulation also reduced the SP content. Milin (117) found that exogenous SP reduces the movement of earthworms to light and vibrational stimulation. The morphodynamic changes indicated that SP's action is to preserve the integrity of the photo- and tactoreceptor cells in the epidermis.

Walaszek *et al.* (118, 119) found less SP in the hypothalamus of rabbits, which had been previously injected with serum from schizophrenic patients. Serum from normal patients produced no change. While glial tumors do not normally contain SP, 13 units/Gm. was found in 1 multiform glioblastoma of a previously irradiated patient (107).

No SP release has been found in the mammalian brain on electrical stimulation (19, 52, 120), but stimulation is reported to release SP in the frog spinal cord (121) and stomach muscles (122–124). Cervical vagotomy increases the SP content of rabbits' small intestine from 2.8 to 7.2 units/Gm., while vagus stimulation significantly decreases the SP content with a return to control levels within 30 min. (125). This is opposite to earlier findings that vagus stimulation increased SP content in the intestine (126–128).

Pernow and Wallensten (92) found the SP content of human intestinal segments was higher when their motility was increased by intraluminar administration of hypertonic glucose. In Hirschsprung's disease the proximal hyperactive part was higher than controls, and the aganglionic and inactive segments of the colon had significantly less SP (91).

PHARMACOLOGICAL EFFECTS

Most studies on the pharmacological actions of SP have used very impure preparations containing many other active substances. The usual

so-called purified samples (250–1000 units/mg.) still contain less than 1% pure SP, while the highly purified SP (50,000–120,000 units/mg.) is extremely labile in aqueous solutions, so many of the reported pharmacological actions are not completely conclusive.

Substance P has a strong stimulatory effect on the mobility of the gastrointestinal tract of most vertebrates, both in isolated segments and *in situ* (1, 21, 50, 129, 130), with a positive correlation between SP content and sensitivity (21). The amplitude and frequency of the peristaltic waves are increased, besides a lower peristaltic threshold, when SP is applied intraluminally or in the bath of isolated intestines of rabbit or guinea pig (4, 131). Substance P restores fatigue or temperature blocked peristaltic activity (4, 132). This response is dependent upon an intact mucous membrane. Intravenous injections of SP markedly increase the segmental and peristaltic movement of the small intestine *in situ* of rabbits and man (129, 131). The increase is observed within a few minutes after start of infusion (50 units/min.) and lasts for 20 min. after ceasing the infusion. In the case of patients with paralytic ileus, SP induced both segmental and peristaltic activity only for the duration of the infusion. Intravenous administration of SP has been also observed to increase the spontaneous movement of the intestinal villi (133). Substance P (50 units/Kg.) injected intra-aortally increased the tonus of the ileocaecal valve of the cat (134). A strong peristaltic response occurs with rhythmic movement of the sphincter ani in the anesthetized dog (28). There was, however, no visible effect on the intestine of mice, with doses to 50,000 units of purified SP (28).

In the guinea pig, intravenous SP has a strong bronchoconstrictor effect (17, 28, 135, 136) but no effect on inhalation (137). No tachyphylaxis occurs (17). Substance P protects against protein induced anaphylactic shock in the guinea pig (138), while plasma levels of SP are independent of occurrence of anaphylaxis induced in dog (139). Euler and Pernow (140, 141) found intraventricular administration of SP (300 units/mg.) produced both tachypnea and hyperpnea in rabbits and cats. Haefely *et al.* (142, 143) found no effect with purified SP (200 or 24,000 units/mg.) on the respiration rate, but did with a crude preparation (20 units/mg.). Substance P given intravenously has no effect on respiration in the cat (142), but subcutaneously produced bradypnea in the guinea pig (8).

Intravenous or intra-arterial infusion of SP causes peripheral vasodilation with an accompanying brief fall in blood pressure (1, 21, 25, 112, 144–146). It is about 100 times more active than acetylcholine and 5 times more active than bradykinin as a vasodilator on a molar basis (28, 50, 147). Bilateral cervical vagotomy has no effect on the depressor response (50, 112). Substance P-evoked pressure increases in the recipient head were abolished by bilateral cervical vagotomy, but not the systemic depressor effect in cross circulation experiments in dogs (148). Intravenous injections of SP in man produced tachycardia and a slight fall in arterial blood pressure. Cardiac output was increased, but not stroke volume, intracardiac, and pulmonary blood pressures (147, 149). An increase in blood flow in skin and muscles occurs within 1 min. after the start of intravenous infusion of SP, and this is coupled with an intense burning sensation in the face and a throbbing head pain. The splanchnic blood flow is, however, not altered. Intra-arterial injections (1 unit/min.) tripled the blood flow in the forearm within seconds, with increased oxygen saturation of both deep and superficial venous blood (147, 150). A transient tachyphylactic effect occurs, so tremendously high doses have only slightly greater effects than a minimum effective dose in mammals. After 15–30 min., identical hemodynamic effects can be reinduced. In the chicken the response is directly related to the amount of SP injected (112). In the conscious dog high doses of SP (7500 units/Kg. i.v.) increase the duration of the depressor effect accompanied by a marked bradycardia which persists after the blood pressure has returned to normal (28). The minimum effective dose in the cat is about 10 times that of other species (21, 28, 50). Circulatory shock could not be induced in the cat, mouse, or man (28, 147). Intraventricular administration of purified SP (24,000 units/mg.) in the cat had no effect on blood pressure (143). No changes in frequency or contractility of the myocardium were found by direct application of SP (28). Substance P has a vasoconstrictor effect on the perfused frog leg (56).

Slow intravenous infusion of an impure SP killed decerebrated mice sooner than normal. The toxic dose is 12,000–18,000 units/Kg. in normal mice. With slow infusion most animals die in convulsions, while rapid injection produces paralysis at death. Physiological saline or inactivated SP controls never produced convulsions (116).

Capillary permeability is significantly increased by small doses of SP in the guinea pig but not in

the rabbit (17, 50, 151–153). Topical application of 0.5–2 ng. of pure SP on the mesenterium of the anesthetized rat shows a clear relaxation of the venule walls followed by a diffused efflux of leukocytes (50). Lewis (154) has also found that SP causes migration and accumulation of leukocytes. Fibrinolysis is inhibited by 20–80 units of SP (155). Cutaneous injections of 10–30 ng. of pure SP produce a burning pain and erythema after a latency of 10–20 sec., which lasts for 10–30 min. (50). A burning sensation occurs when it is applied to an exposed blister base (17, 156). Intra-arterial, but not intravenous, injections of SP induce visceral pain in dogs (157).

An antidiuretic and milk ejection effect are observed, but only in doses having a marked depressor action (17). The ACTH releasing ability of SP is due to an impurity (158, 159). It has no epinephrine releasing action on the adrenal medulla (160). Substance P inhibits oxytocin induced contractions of the rat uterus *in vivo* (161).

Ten minutes after an intraperitoneal dose (3000 units/Kg.), SP increases in concentration in the brain and spinal cord of the rat, reaching a maximum in 30–40 min., and after 60–120 min. returns to normal or slightly below (116). This time course for SP uptake could account partially for many observations on the effects of exogenous SP alone and with central acting drugs on the nervous system. Sedation, *i.e.*, a reduction in spontaneous and induced motor activity, is the most often reported behavioral effect of administered SP (8, 10, 12, 13, 141, 162–165). Oral automatisms were often provoked. All of these observations are somewhat in question, as Haefely *et al.* (143) found no observable behavioral effects after intraventricular injection of purified SP in the cat, but did observe sedation with crude extracts. They showed that ammonium sulfate in the concentrations contained in the crude extracts would produce sedation. This observation does not explain, however, the chymotrypsin inactivated SP controls used by other authors which produced no effect. Also the same investigators (19, 28) reported that 7500 units/Kg. of pure SP produced sedation in the unanesthetized dog. In mice no clear effect was observed with doses up to 1000 units per animal given intravenously or intracerebrally. With 5000 units, spontaneous, but not induced, activity was reduced (28).

Ganglionic transmission in the superior cervical ganglion of the cat was not affected by high doses of the purified SP (19), although a dual effect of potentiation and inhibition has been reported using impure extracts (140, 166, 167). Caspers (10, 168, 169) correlated behavioral with bioelectrical changes in the unanesthetized rat after administration of an impure SP extract. The EEG and d.c. potential patterns of the cortex were those of natural sleep following intraperitoneal and topical SP application. Dendritic potentials are increased in voltage and duration. The reactivity of the animals to acoustic stimulation was easily depressed, while tactile stimulation was more resistant. Conditioned responses were more resistant to SP than unconditioned ones. Besides a hyperpolarizing effect at the cortex, Caspers found SP reduced the unit discharge in the brain stem reticular formation, but had little effect at the thalamic relay nuclei. Schneiderman *et al.* (170), however, found SP (75 units/Kg. i.v.) decreases the threshold of the thalamocortical evoked potential in the rabbit, depressing the amplitude of the first surface-positive component and enhancing the second and third surface-negative components. Substance P increased cortical Δ activity and the number of spindles. The onset of changes occurred within 5 min. of administration, reaching a maximum within 15, and lasting about 1 hr. Cortical activation and hippocampal synchronization have been found with intracarotid injection (7) or topical application (171) of SP, but are probably a secondary effect produced by blood pressure depression (10).

Stern *et al.* (172) found an inhibitory influence of SP on polysynaptic, but not monosynaptic reflexes in the cat, while Kissel and Domino (173) found no effect on either. No effect was found on polysynaptic reflexes in the frog (121). Substance P had no influence on conditioned reflexes, discrimination tests, and maze performance of the rat (174).

Substance P produces no change in the dorsal root potential (175–177) or the amplitude variation in the electric knife fish (*G. eigenmannia*) (11). Substance P stimulates the sensory nerve endings in the rabbit ear (178).

DRUG INTERACTION

The failure of atropine to block the contracting action of SP on the intestine led to its discovery (1). Since then the interaction of many compounds with SP on isolated intestinal tissue has been determined, primarily to find a specific antagonist. Only two reasonably specific antagonists have been found: cystine-di-β-naphthylamide and trimethaphane-*d*-camphorsulfonate (47, 48). Drugs which exert no inhibitory or an unspecific effect on the SP action on the isolated guinea pig ileum are as follows: adenosine,

adermine, γ-aminobutyric acid, *p*-aminobenzoic acid, 1-AMP, 2-AMP, 5-AMP, antimit, antazoline phosphate, antipernicin, atropine, ATP, antihistamines, camylofine, azulen benzoquinone, biotin, 2-bromo-*d*-lysergic acid diethylamide, hyoscine butylbromide, bulbocapnine, barbital, bradykinin, caffeine, catechol, chlorpromazine, ε-amino-*n*-caproic acid, citric acid, cocaine, codeine, cysteine, cyanocobalamin, dehydrocholate, deoxyribonucleic acid, dihydroergotamine, dichloroisoproterenol, buphenine, eutison, glutathione, heparin, hexamethonium, histamine, hydrochloric acid, hydrazines, pentylenetetrazol riboflavin, *d*-lysergic acid diethylamide, morphine, iproniazid, kallidine, procaine hydrochloride, NaF, NaCN, mephenesin, meprobamate, 1,2-naphthoquinone, 1,4-naphthoquinone, methysergide, α-naphthylamine, β-naphthylamine, *n*-(naphthyl)-methylenediamine, narceine, dihydralazine, nicotine, oxytocin, patulin, phenacemide, phenoxybenzamine, polyethylene sulfate, protamine, pyridoxalphosphate, pyridoxine, quiloflex, reserpine, quinine, 8-hydroxyquinoline, 6-ethoxy-7-methoxy-1-(3′,4′-diethoxybenzyl)-3-methyl isoquinoline, renin, scopolamine, prenylamine, semicarbazide, sparteine, strychnine, tetraethylammonium bromide, thiamine, thioglycerin, thiosemicarbazide, trimethadione, tripelennamine, urea, vasopressin (1, 21, 40, 41, 47, 48, 50, 96, 179–184).

A few drugs, while not specific, inhibit the SP response, as 5-adenylic acid, noscapine, acetyl thiamine, adrenochrome, papaverine, trasentin-6H, D-tubocurarine, gallamine, trihexyphenidyl hydrochloride, ibogaine, harmoline (47, 50, 182–186).

While LSD potentiates the response of SP on the guinea pig ileum (187–189), BOL-148 has an inhibitory effect (183). Succinylcholine and psilocybin also potentiate the effect of SP (185, 190). Substance P potentiates the intestinal response to acetylcholine and nicotine with slight depression of serotonin and no effect on histamine or γ-aminobutyric acid responses (184, 191).

Hexamethonium, morphine, and morphine-like analgesics inhibit the stimulating action of SP on the peristaltic reflex (132, 192). Substance P is able to restore peristalsis when abolished by either external or internal application of serotonin or tubocurarine (4, 5, 193) but not bradykinin (194). Purified SP has a potentiating effect on epinephrine induced contractions of the nictitating membrane (19).

Morphine, physostigmine, or atropine injected 30 min. before sacrifice produced no change in the SP content of the small intestine of the rat (181). Physostigmine significantly decreases the SP content of the rabbit's intestine, while hexamethonium, chlorisondamine, atropine, reserpine, chlorpromazine, *p*-hydroxymercuribenzoate, and nicotine have no definite effect (125). Intraperitoneal injections of 100 units/Kg. of SP for 3 days significantly increases the serotonin content of the rat's ileum and stomach, but not of the spleen. Intraluminal application for 30 min. of SP (60–80 units) doubles the serotonin content of the isolated guinea pig ileum (132, 195).

Acetylsalicylate, mepyramine, atropine, or lysergic acid diethylamide do not inhibit the bronchoconstrictor response of SP in the guinea pig (135, 136).

Atropine, ganglionic blocking agents, antihistamines, or guanethidine have no effect on circulatory responses of SP in mammals (21, 144, 150, 196). Dihydroergotamine, caffeine, mecamylamine, chlorisondamine, hexamethonium, and methysergide diminish the SP arterial depressor response in the intact chicken, but have no vascular effect on the isolated chicken wing preparation or cat blood pressure. The response of these drugs is nonspecific. Atropine, *p*-bromdylamine, LSD, 1-benzyl-2-methyl-5-hydroxytryptamine (BAS-phenol), phenethylamine, harmaline, ibogaine, morphine, and reserpine have no or slight antagonistic effects in the intact chicken (112). Mepyramine reduces SP-induced increased capillary permeability (17).

The effect of drugs on SP concentration in the central nervous system has been investigated by several authors with no unanimity of results. Stern (116) found that strychnine (0.5 mg./Kg. i.p.) after 1–3 hr. reduced the amount of SP in the brain and spinal cord of the rat, while procaine (50 mg./Kg. i.p.) increased the amount. Mephenesin (150 mg./Kg. i.p.) had no effect at 1 hr. and caused only a slight reduction at 3 hr. Allergic encephalitis does not change the SP concentration. Haefely (197) found no change in content in the rat's brain after giving reserpine, barbiturates, chlorpromazine, amphetamine, and LSD. Stern and Kocic-Mitrovic (198), however, found a large increase in SP in the brain of the rat after reserpine treatment, but no change in concentration after phenobarbital, chlorpromazine, meprobamate, mephenesin, or syrosingopine. No change in the rat's brain SP content occurred after giving neostigmine, epinephrine, norepinephrine, serotonin, histamine, factor I, or γ-aminobutyric acid (199). Capsaicine, given subcutaneously, significantly reduces SP in the spinal cord of the rat, but not in the brain. *p*-Bromophenylacetyl urea had no

effect on the spinal cord concentration (111). Cocaine increases SP content in the dorsal roots proximally to site of topical application (200).

No change in SP brain concentration in mice was found after LSD, reserpine, morphine, chloroform, ether, chlorpromazine, barbital, insulin, diphenylhydantoin, pentylenetetrazol, or trihexyphenidyl hydrochloride treatment (182). Zetler and Ohnesorge (201) observed an increase in SP in the mouse brain after amphetamine and morphine and a decrease after chloroform, urethan, and phenobarbital, while LSD produced no change. While no change in total brain concentration was found after picrotoxin or LSD, if the "free" and "bound," *i.e.*, easy or difficult to extract portions were separately determined; picrotoxin increased the "free" and lowered the "bound" amounts of SP and LSD decreased the "bound" form.

Physostigmine decreased the concentration of SP in the rabbit brain, while reserpine, chlorpromazine, and *p*-hydroxymercuribenzoate had no effect (202).

In anesthetized dogs there was no change in the SP content in the hypothalamus and caudate nucleus after amphetamine, ephedrine, insulin, β-tetrahydronaphthylamine, caffeine, and reserpine (94). Reserpine has no effect on SP content in peripheral nerves of the dog (76).

The effect of exogenous SP on the action of central acting drugs is no more conclusive than the effect of these drugs on the endogenous concentration of SP. Zetler (8, 203, 204) found impure SP protected mice against strychnine and picrotoxin convulsions. It prevented the central effects of harmine and methamphetamine and prolonged hexobarbital sleeping time and bulbocapnine catelepsy. Substance P also antagonized morphine analgesia and caused hyperalgesia, potentiated caffeine convulsions, and lowered the threshold to painful stimuli. It had no effect on seizures induced by electroshock, ammonium acetate, nicotine, or pentylenetetrazole. These drugs were tested after 15–30 min. of SP treatment. However, Stern *et al.* (205, 206) found that a slightly purified SP sample potentiated strychnine convulsion, and shortened hexobarbital sleeping time—the opposite to the impure sample, but still antagonized morphine analgesia. Substance P acted as an antagonist to morphine analgesia in the mouse writhing tests (207), while Bonta *et al.* (208) found a strychnine antagonist in their impure SP preparation, but it had no effect on hexobarbital sleeping time or morphine analgesia. They found the strychnine antagonistic property also in other SP-free fractions. Pure SP had no strychnine antagonistic ability (50). Gaddum *et al.* (209) later separated an antistrychnine substance from the crude SP. Kapek (210) found that intracerebral injections of SP increased seizure thresholds to convulsions induced by pentylenetetrazol, strychnine, and electroshock. Stern *et al.* (172, 211, 212) found SP has an antagonistic effect against tetanus toxin, and iminodipropionitrile, and a synergistic effect with mephenesin and meprobamate. Substance P inhibits epileptic attacks induced by audiogenic stress in mice (213).

After observing the changes in concentration with time in the central nervous system after giving exogenous SP, Stern (116) re-examined several drugs to determine the influence of time of administration on the results, using an impure SP sample. Zetler's results were confirmed when SP was given 15 min. before administration of the other drugs. Strychnine, picrotoxin, caffeine, or morphine actions are, however, potentiated when administered 60 min. after SP and hexobarbital sleeping time is shortened. The action of chloral hydrate is lengthened when administered 15 or 60 min. after SP. Bulbocapnine catalepsy is lengthened at all times. Audiogenic stress is not inhibited after 60 min., but is at 15 min. Harmine tremor is antagonized by SP at all times tested. Pentylenetetrazole convulsions are only slightly influenced by SP after 60 min. Substance P not only antagonized morphine analgesia, but also suppressed the withdrawal symptoms in addicted rats. Impure SP given 60 min. prior shortened morphine, pethantine, and heptadon analgesia. It did not act against mephenesin paralysis after either 15 or 60 min. Substance P shows protection during the first 30 min. to strychnine convulsions and hexobarbital narcosis, then excitement for the next 1.5 hr., and by the second hour a return to the central behavior pattern.

Substance P had no influence on cocaine or procaine local anesthesia. The lethal dose of slow intravenous infusion of SP after LSD administration in the decorticated mouse is one-third to one-fourth of that in the nontreated normal animal (116). Both crude and pure SP (10,000 units/mg.) enhance the fourth dorsal root potential after LSD administration (175–177). The amplitude variations of the electric knife fish are significantly decreased following LSD and SP administration (11). Local application of γ-aminobutyric acid antagonizes the effect of SP on the d.c. potential in the cortex (168).

CONCLUSIONS

The chemistry, distribution, physiology, and pharmacology of SP has been reviewed. The presence of SP in all cordates, the characteristic

distribution in the gastrointestinal tract and nervous system, the changes in endogenous concentration with different functional states, and the high pharmacological activity of the pure substance indicate teleologically that SP has a definite physiological function. However, this function is still largely obscure, particularly within the nervous system. This makes SP an interesting and important physiological and pharmacological problem.

REFERENCES

(1) Euler, U. S. v., and Gaddum, J. H., *J. Physiol. (London)*, **72,** 74(1931).
(2) Euler, U. S. v., *Arch. Exptl. Pathol. Pharmakol.*, **181,** 181(1936).
(3) Pernow, B., *Acta Physiol. Scand.*, **24,** 97(1951).
(4) Beleslin, D., and Varagic, V., *Brit. J. Pharmacol.*, **13,** 321(1958).
(5) Beleslin, D., and Varagic, V., *J. Pharm. Pharmacol.*, **11,** 99(1958).
(6) Lembeck, F., *Arch. Exptl. Pathol. Pharmakol.*, **219,** 197(1953).
(7) Lechner, H., and Lembeck, F., *ibid.*, **234,** 419(1958).
(8) Zetler, G., *ibid.*, **228,** 513(1956).
(9) Zetler, G., in "Polypeptides Which Affect Smooth Muscles and Blood Vessels," Schachter, M., ed., Pergamon Press, New York, N. Y., 1960, p. 179.
(10) Caspers, H., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 29(1961).
(11) Krivoy, W. A., *et al.*, *Ann. N. Y. Acad. Sci.*, **104,** 312(1963).
(12) Stern, P., and Milin, R., *Proc. Soc. Exptl. Biol. Med.*, **101,** 298(1959).
(13) Stern, P., and Dobric, V., in "Psychotropic Drugs," Garattini, S., and Ghetti, V., eds., Elsevier Publishing Co., New York, N. Y., 1957, p. 448.
(14) Umrath, K., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 23(1961).
(15) Umrath, K., *Arch. Ges. Physiol.*, **262,** 368(1956).
(16) Umrath, K., and Bohn, H., *ibid.*, **277,** 523(1963).
(17) Bisset, G. W., and Lewis, G. P., *Brit. J. Pharmacol.*, **19,** 168(1962).
(18) Starke, K., *Arch. Exptl. Pathol. Pharmakol.*, **247,** 149(1964).
(19) Haefely, W., and Hurlimann, A., *Experientia*, **18,** 297(1962).
(20) Lembeck, F., and Zetler, G., *Intern. Rev. Neurobiol.*, **4,** 157(1962).
(21) Pernow, B., *Acta Physiol. Scand., Suppl.* 105, **21,** 1 (1953).
(22) Pernow, B., *Z. Vitamin-Hormon-Fermentforsch.*, **7,** 59(1955).
(23) Zetler, G., *Arch. Exptl. Pathol. Pharmakol.*, **245,** 263(1963).
(24) Anand, N., and Khosla, M. C., *J. Sci. Ind. Res. (India)*, **23,** 8(1964).
(25) Erspamer, V., *Ann. Rev. Pharmacol.*, **1,** 175(1961).
(26) Michaud, J., *Presse Med.*, **72,** 2693(1964).
(27) Inouye, A., and Kataoka, K., *Tampakushitsu Kakusan Kosa*, **9,** 394(1964).
(28) Vogler, K., *et al.*, *Ann. N. Y. Acad. Sci.*, **104,** 378 (1963).
(29) Boissonnas, R. A., *et al.*, *ibid.*, **104,** 376(1963).
(30) Zuber, H., *ibid.*, **104,** 391(1963).
(31) Zuber, H., and Jaques, R., *Angew. Chem. Intern. Ed. Engl.*, **1,** 160(1962).
(32) Franz, J., *et al.*, *Helv. Chim. Acta*, **44,** 881(1961).
(33) Cleugh, J., and Gaddum, J. H., *Experientia*, **19,** 72(1963).
(34) Euler, U. S. v., *Acta Physiol. Scand.*, **4,** 373(1942).
(35) Zetler, G., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 89(1961).
(36) Cleugh, J., *et al.*, *J. Physiol (London)*, **170,** 69(1964).
(37) Pernow, B., *Methods Med. Res.*, **9,** 220(1961).
(38) Laszlo, I., *J. Physiol. (London)*, **153,** 69P(1960).
(39) Laszlo, I., *Brit. J. Pharmacol.*, **20,** 449(1963).
(40) Haley, T. J., *et al.*, unpublished data.
(41) Gaddum, J. H., *J. Physiol. (London)*, **119,** 363(1953).
(42) Brownlee, G., and Harry, J., *Brit. J. Pharmacol.*, **21,** 544(1963).
(43) Innes, I. R., *et al.*, *J. Physiol. (London)*, **137,** 396 (1957).
(44) Blair, M. R., and Clark, B. B., *J. Pharmacol. Exptl. Therap.*, **117,** 467(1956).
(45) Walaszek, E. J., and Dyer, D. C., *Biochem. Pharmacol.*, **14,** 1401(1965).
(46) Libonati, M., and Segre, G., *Arch. Exptl. Pathol. Pharmakol.*, **240,** 14(1960).
(47) Stern, P., and Hukovic, S., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 83(1961).
(48) Hukovic, S., and Stern, P., *Arch. Exptl. Pathol. Pharmakol.*, **245,** 281(1963).
(49) Cleugh, J., *et al.*, *Brit. J. Pharmacol.*, **17,** 144(1961).
(50) Jaques, R., *Arch. Exptl. Pathol. Pharmakol.*, **245,** 275(1963).
(51) Gaddum, J. H., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 7(1961).
(52) Gaddum, J. H., and Szerb, J. C., *Brit. J. Pharmacol.*, **17,** 451(1961).
(53) Pernow, B., and Rocha e Silva, M., *Acta Physiol. Scand.*, **34,** 59(1955).
(54) Franz, J., *et al.*, *Proc. Sci. Soc. Bosnia Herz.*, **1,** 99(1961).
(55) Euler, U. S. v., *Skand. Arch. Physiol.*, **73,** 142 (1936).
(56) Amin, A. H., *et al.*, *J. Physiol. (London)*, **126,** 596 (1954).
(57) Leach, E., *ibid.*, **149,** 34P(1959).
(58) Lembeck, F., *et al.*, *Biochem. Pharmacol.*, **14,** 1394 (1965).
(59) Zetler, G., *Arch. Exptl. Pathol. Pharmakol.*, **241,** 544(1961).
(60) *Ibid.*, **242,** 330(1961).
(61) *Ibid.*, **243,** 360(1962).
(62) *Ibid.*, **246,** 504(1964).
(63) Zetler, G., *Ann. N. Y. Acad. Sci.*, **104,** 416(1963).
(64) Kataoka, K., *23rd Intern. Congr. Physiol. Sci.*, **1965,** 408.
(65) Eliasson, R., *et al.*, *Brit. J. Pharmacol.*, **11,** 137 (1956).
(66) Eber, O., and Lembeck, F., *Arch. Exptl. Pathol. Pharmakol.*, **229,** 139(1956).
(67) Hooper, K. C., *Biochem. J.*, **83,** 511(1962).
(68) *Ibid.*, **88,** 398(1963).
(69) Gullbring, B., *Acta Physiol. Scand.*, **6,** 246(1943).
(70) Pernow, B., *ibid.*, **34,** 296(1955).
(71) Matussek, N., *Z. Physiol. Chem.*, **316,** 241(1959).
(72) Arvidsson, U. B., *et al.*, *Acta Physiol. Scand.*, **35,** 338(1956).
(73) Stern, P., *et al.*, *Proc. Sci. Soc. Bosnia Herz.*, **1,** 139(1961).
(74) Kocic-Mitrovic, D., *ibid.*, **1,** 113(1961).
(75) Tsujioka, T., *Physiol. Soc. Japan*, **20,** 662(1958).
(76) Euler, U. S. v., *Ann. N. Y. Acad. Sci.*, **104,** 449 (1963).
(77) Gaddum, J. H., and Schild, H., *J. Physiol. (London)*, **83,** 1(1934).
(78) Zetler, G., and Schlosser, L., *Arch. Exptl. Pathol. Pharmakol.*, **224,** 159(1955).
(79) Correale, P., *Arch. Ital. Sci. Farmacol.*, **6,** 223 (1956).
(80) Correale, P., *Arch. Intern. Pharmacodyn.*, **119,** 435(1959).
(81) Grabner, K., *et al.*, *Arch. Exptl. Pathol. Pharmakol.*, **236,** 331(1959).
(82) Euler, U. S. v., and Ostlund, E., *Brit. J. Pharmacol.*, **11,** 323(1956).
(83) Dahlstedt, E., *et al.*, *Acta Physiol. Scand.*, **47,** 124(1959).
(84) Gaddum, J. H., in "Polypeptides Which Affect Smooth Muscles and Blood Vessels," Schachter, M., ed., Pergamon Press, London, England, 1960, p. 163.
(85) Pernow, B., in "Polypeptides Which Stimulate Plain Muscle," Gaddum, J. H., ed., Livingston, London, England, 1955, p. 28.
(86) Laszlo, I., *J. Physiol. (London)*, **172,** 30P(1964).
(87) Euler, U. S. v., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 103(1961).
(88) Lembeck, F., and Petschke, B., in *Intern. Rev. Neurobiol.*, **4,** 157(1962).
(89) Zetler, G., and Schlosser, L., *Naturwissenschaften*, **41,** 46(1954).
(90) Arnautovic, I., *et al.*, *Med. Exptl.*, **9,** 233(1963).
(91) Ehrenpreis, T., and Pernow, B., *Acta Physiol. Scand.*, **27,** 380(1953).
(92) Pernow, B., and Wallensten, S., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 57(1961).
(93) Zetler, G., and Schlosser, L., *Naturwissenschaften* **40,** 559(1953).
(94) Paasonen, M. K., and Vogt, M., *J. Physiol. (London)*, **131,** 617(1956).
(95) Kopera, H., and Lazarini, W., *Arch. Exptl. Pathol. Pharmacol.*, **219,** 214(1953).
(96) Douglas, W. W., *et al.*, *J. Physiol. (London)*, **115,** 163(1951).
(97) Duner, H., *et al.*, *Acta Physiol. Scand.*, **31,** 113 (1954).
(98) Umrath, K., *Arch. Ges. Physiol.*, **258,** 230(1953).
(99) Kataoka, K., *Japan. J. Physiol.*, **12,** 81(1962).
(100) Inouye, A., *et al.*, *Biochim. Biophys. Acta*, **71,** 491(1963).
(101) Inouye, A., *et al.*, *Nature*, **194,** 585(1962).
(102) Ryall, R. W., *ibid.*, **196,** 680(1962).
(103) Ryall, R. W., *Biochem. Pharmacol.*, **11,** 1234(1962).
(104) Ryall, R. W., *J. Neurochem.*, **11,** 131(1964).
(105) Lembeck, F., and Holasek, A., *Arch. Exptl. Pathol. Pharmakol.*, **238,** 542(1960).
(106) Euler, U. S. v., and Lishajko, F., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 109(1961).
(107) Grabner, K., and Lembeck, F., *Arch. Exptl. Pathol. Pharmakol.*, **240,** 157(1960).
(108) Andrews, T. M., and Holton, P., *J. Physiol. (London)*, **143,** 45P(1958).
(109) Holton, P., *ibid.*, **149,** 35P(1959).
(110) Holton, P., in "Polypeptides Which Affect Smooth Muscles and Blood Vessels," Schachter, M., ed., Pergamon Press, New York, N. Y., 1960, p. 192.
(111) Gasparovic, K., *et al.*, *Med. Exptl.*, **10,** 303(1964).

(112) Bunag, R. D., and Walaszek, E. J., *Ann. N. Y. Acad. Sci.*, **104,** 437(1963).
(113) Lembeck, F., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 97 (1961).
(114) Stern, P., and Kocic-Mitrovic, D., *Naturwissenschaften*, **45,** 213(1958).
(115) Kocic-Mitrovic, D., *Arch. Exptl. Pathol. Pharmakol.*, **236,** 37(1959).
(116) Stern, P., *Ann. N. Y. Acad. Sci.*, **104,** 403(1963).
(117) Milin, R., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 75(1961).
(118) Walaszek, E. J., *et al., Federation Proc.*, **17,** 416 (1958).
(119) Walaszek, E. J., *Intern. Rev. Neurobiol.*, **2,** 137 (1960).
(120) Hilton, S. M., and Schain, P. J., *J. Physiol. (London)*, **157,** 46P(1961).
(121) Angelucci, L., *Brit. J. Pharmacol.*, **11,** 161(1956).
(122) Singh, I., *Arch. Intern. Pharmacodyn.*, **143,** 138 (1963).
(123) *Ibid.*, **146,** 350(1963).
(124) Singh, I., *Arch. Intern. Physiol. Biochem.*, **71,** 371(1963).
(125) Radmanovic, B., *Acta Physiol. Scand.*, **61,** 272 (1964).
(126) Vogt, W., *Arch. Exptl. Pathol. Pharmakol.*, **206,** 1(1949).
(127) Engstedt, L., in *Acta Physiol. Scand., Suppl. 105,* **29,** 44 (1953).
(128) Euler, U. S. v., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 52(1961).
(129) Liljedahl, S. O., *et al., Scand. J. Clin. Lab. Invest.*, **10,** 16(1958).
(130) Euler, U. S. v., and Ostlund, E., *Acta Physiol. Scand.*, **38,** 364(1956).
(131) Gernandt, B., *ibid.*, **3,** 270(1942).
(132) Varagic, V., *et al., Proc. Sci. Soc. Bosnia Herz.*, **1,** 65(1961).
(133) Ludany, G., *et al., Arch. Ges. Physiol.*, **270,** 499 (1960).
(134) Grabner, K., and Lembeck, F., in *Intern. Rev. Neurobiol.*, **4,** 194(1962).
(135) Bhoolar, K. D., *et al., Brit. J. Pharmacol.*, **19,** 190(1962).
(136) Collier, H. O. J., *Ann. N. Y. Acad. Sci.*, **104,** 290 (1963).
(137) Stern, P., *ibid.*, **104,** 297(1963).
(138) Stern, P., and Hukcevic, S., *Acta Allergol. Symp.*, **7,** 370(1960).
(139) Suzuki, K., and Arai, Y., *Arerugi*, **10,** 253(1961).
(140) Euler, U. S. v., and Pernow, B., *Acta Physiol. Scand.*, **36,** 265(1956).
(141) Euler, U. S. v., and Pernow, B., *Nature*, **174,** 184 (1954).
(142) Haefely, W., *Arch. Exptl. Pathol. Pharmakol.*, **245,** 273(1963).
(143) Haefely, W., *et al., Med. Exptl.*, **7,** 245(1962).
(144) Pernow, B., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 45 (1961).
(145) Holton, F. A., and Holton, P., *J. Physiol. (London)*, **118,** 310(1952).
(146) Stürmer, E., and Franz, J., *Med. Exptl.*, **5,** 37(1961).
(147) Pernow, B., *Ann. N. Y. Acad. Sci.*, **104,** 393(1963).
(148) Kato, H., and Buckley, J. P., *J. Pharmacol. Exptl. Therap.*, **148,** 298(1965).
(149) Duner, H., and Pernow, B., *Acta Physiol. Scand.*, **49,** 261(1960).
(150) Lofstrom, B., *et al., Acta Physiol. Scand.*, **63,** 311 (1965).
(151) Bianchini, P., *Farmaco (Pavia) Ed. Sci.*, **18,** 756 (1963).
(152) Lembeck, F., and Starke, K., *Nature*, **199,** 1295 (1963).
(153) Zweifach, B. W., *Biochem. Pharmacol.*, **14,** 1402 (1965).
(154) Lewis, G. P., *Ann. N. Y. Acad. Sci.*, **104,** 236 (1961).
(155) Bianchini, P., *Farmaco (Pavia) Ed. Sci.*, **18,** 763 (1963).
(156) Armstrong, D., *et al., Nature*, **174,** 791(1954).
(157) Potter, G. D., *et al., ibid.*, **193,** 983(1962).
(158) Swingle, W. W., *et al., Proc. Soc. Exptl. Biol. Med.*, **92,** 594(1956).
(159) Guillemin, R., *et al., Endocrinology*, **60,** 488(1957).
(160) Feldberg, W., and Lewis, G. P., *J. Physiol. (London)*, **171,** 98(1964).
(161) Bianchini, P., *Farmaco (Pavia) Ed. Sci.*, **18,** 773 (1963).
(162) Stern, P., and Hukovic, S., *Naturwissenschaften*, **45,** 626(1958).
(163) Stern, P., *Biochem. Pharmacol.*, **14,** 1399(1965).
(164) Pernow, B., and Euler, U. S. v., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 53(1961).
(165) Stern, P., and Dobric, V., *Naturwissenschaften*, **44,** 517(1957).
(166) Beleslin, D., and Radmanovic, B. Z., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 61(1961).
(167) Beleslin, D., *et al., Brit. J. Pharmacol.*, **15,** 10(1960).
(168) Caspers, H., *Med. Exptl.*, **2,** 198(1960).
(169) Caspers, H., and Stern, P., *Arch. Ges. Physiol.*, **273,** 94(1961).
(170) Schniederman, N., *et al., Experientia*, **21,** 596 (1965).
(171) Crepax, P., and Infantellini, F., *Arch. Sci. Biol. (Bologna)*, **40,** 147(1956).
(172) Stern, P., *et al., Arch. Intern. Pharmacodyn.*, **112,** 102(1957).
(173) Kissel, J. W., and Domino, E., *J. Pharmacol. Exptl. Therap.*, **125,** 168(1959).
(174) Stern, P., *Acta Physiol. Pharmacol. Neerl.*, **8,** 10 (1959).
(175) Krivoy, W. A., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 131(1961).
(176) Krivoy, W. A., *Brit. J. Pharmacol.*, **16,** 253(1961).
(177) Krivoy, W. A., and Krueger, D., *Experientia*, **19,** 366(1963).
(178) Lembeck, F., *Arch. Exptl. Pathol. Pharmakol.*, **230,** 1(1957).
(179) Eliasson, R., *Experientia*, **14,** 460(1958).
(180) Lewis, G. P., *Brit. J. Pharmacol.*, **15,** 425(1960).
(181) Lembeck, F., *Proc. Sci. Soc. Bosnia Herz.*, **1,** 97 (1961).
(182) Laszlo, I., *Brit. J. Pharmacol.*, **21,** 113(1963).
(183) Walaszek, E. J., *et al., Ann. N. Y. Acad. Sci.*, **104,** 281(1963).
(184) Inouye, A., *et al., Japan. J. Physiol.*, **11,** 319(1961).
(185) Radmanovic, B. Z., *Acta Med. Iugoslav.*, **15,** 251 (1961).
(186) Stern, P., *et al., Naturwissenschaften*, **43,** 162(1956).
(187) Smith, C. M., and Walaszek, E. J., *Federation Proc.*, **19,** 264(1960).
(188) Smith, C. M., and Walaszek, E. J., *Arch. Intern. Pharmacodyn.*, **138,** 429(1962).
(189) Krivoy, W. A., *Brit. J. Pharmacol.*, **12,** 361(1957).
(190) Vukcevic, S., *Bull. Sci. Conseil Acad. RPF Yougoslavie*, **7,** 103(1962).
(191) Beleslin, D., and Varagic, V., *Arch. Intern. Pharmacodyn.*, **126,** 321(1960).
(192) Medakovic, M., and Radmanovic, B., *ibid.*, **122,** 428(1959).
(193) Radmanovic, B. Z., *Acta Med. Iugoslav.*, **15,** 144 (1961).
(194) Beleslin, D., *et al., Arch. Intern. Pharmacodyn.*, **147,** 43(1964).
(195) Beleslin, D., and Varagic, V., *Experientia*, **15,** 186(1959).
(196) Beck, L., *Federation Proc.*, **24,** 1298(1965).
(197) Haefely, W., *Ann. N. Y. Acad. Sci.*, **104,** 415 (1963).
(198) Stern, P., and Kocic-Mitrovic, D., *Arch. Exptl. Pathol. Pharmakol.*, **238,** 57(1960).
(199) Serafimov, N., and Stern, P., *Arch. Intern. Physiol. Biochem.*, **66,** 653(1958).
(200) Serafimov, N., in Stern, P., *Ann. N. Y. Acad. Sci.*, **104,** 403(1963).
(201) Zetler, G., and Ohnesorge, G., *Arch. Exptl. Pathol. Pharmakol.*, **231,** 199(1957).
(202) Radmanovic, B., *Nature*, **204,** 1205(1964).
(203) Zetler, G., *Arch. Exptl. Pathol. Pharmakol.*, **229,** 148(1956).
(204) *Ibid.*, **237,** 11(1959).
(205) Stern, P., and Hukovic, S., *Med. Exptl.*, **2,** 1(1960).
(206) Hukovic, S., *et al., Proc. Sci. Soc. Bosnia Herz.*, **1,** 15(1961).
(207) Przic, R., *ibid.*, **1,** 71(1961).
(208) Bonta, I. L., *et al., Acta Physiol Pharmacol. Neerl.*, **10,** 114(1961).
(209) Gaddum, J. H., *et al., J. Physiol. (London)*, **172,** 207 (1964).
(210) Kapek, R., *Proc. Intern. Pharmacol.*, **9,** 61(1962).
(211) Stern, P., and Hukovic, S., *Naturwissenschaften*, **43,** 538(1956).
(212) Stern, P., *et al., Acta Med. Iugoslav.*, **12,** 153(1958).
(213) Stern, P., and Lekovic, D., *Acta Pharm. Jugoslav.*, **11,** 11(1961).