# NATURALLY OCCURRING LIPID-SOLUBLE ACIDS OF PHARMACOLOGICAL INTEREST

W. VOGT[1]

*Medizinische Forschungsanstalt der Max-Planck-Gesellschaft zur Förderung der Wissenschaften E.V., Division of Pharmacology, Göttingen, Germany*

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## I. INTRODUCTION

This review deals with a series of pharmacologically active compounds which occur in animal tissues or fluids and which have two features in common: they are acidic in nature and they are—at least in the free acid state—soluble in fat solvents. Some of them were probably present in biological extracts assayed for substances already characterized such as histamine and acetylcholine. In such experiments these lipid-soluble acids were regarded as no more than non-specific impurities. Their presence might explain some of the reported discrepancies between the action of pure amines and of the same amines present in biological fluids.

The main reason why the acidic substances have not been recognized in the past is probably owing to their peculiar physico-chemical behaviour. Classical chemical procedures for separation, purification and identification of substances, such as selective precipitation, extraction or crystallization greatly helped in the study of the alkaloids and the biologically active amines. Applying such procedures to lipid material is difficult and becomes even more so when handling minute amounts of active material present in a large excess of contaminants. It is only since modern analytical and preparative methods, *e.g.*, chromatography and countercurrent distribution, have become available that considerable progress has been made in the study of the pharmacologically active lipid-soluble acids. Nevertheless, our knowledge about this group is still scanty compared with that

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of other substances of biological interest. It is hoped that the present review will stimulate and aid further work in this field.

The various substances have been arranged partly in the order of their detection, and partly according to their chemical nature. Essential fatty acids as such are not included. Their vitamin-like function can at present not be understood in terms of pharmacological actions. For references see Boyer (18). A short review on lipid-soluble acids has already been given (87).

## II. DESCRIPTION OF THE INDIVIDUAL LIPID-SOLUBLE ACIDS

### *A. Prostaglandin and vesiglandin*

PROSTAGLANDIN. It has long been known that extracts of human prostate gland and seminal fluid have smooth muscle-stimulating and depressor activities. These actions are mainly due to a principle which was shown by Goldblatt (41) to be different from other known tissue constituents with similar actions. The active substance was called prostaglandin by Euler, who recognized its acidic nature (28). This term will be used also for Goldblatt's substance since it is apparently the same compound (29).

*1. Physical and chemical properties.* In Euler's laboratory prostaglandin was found during investigations on the occurrence of substance P (27); in contrast to substance P, it was soluble in organic solvents and had acid properties (28, 29).

*Stability.* Prostaglandin is stable to boiling in aqueous solution from pH 3 to 9 (25). The stability on heating in alkali is dependent on the time of boiling and on the alkalinity (29, 41). Bromine (29) and catalytic hydrogenation (14) destroy the activity.

*Solubility.* In the free acid state prostaglandin is slightly soluble in water, and dissolves readily in alcohol, acetone, ether and chloroform (29, 30). From this property one would expect a partition coefficient of $>1$, *e.g.*, for distribution between ether and water. This is, however, not certain since 10 to 20 volumes of ether have been used for the extraction of prostaglandin from an acidified aqueous solution (29, 30). At alkaline pH prostaglandin cannot be extracted from aqueous solution with organic solvents. Alkali and alkaline earth salts of prostaglandin are water-soluble. Heavy metal ions form salts which are insoluble in water but soluble in alcohol (30). As would be expected of an acid, prostaglandin is not precipitated by base-precipitating reagents such as phosphotungstic acid or Reinecke salt.

*Other properties.* Prostaglandin can be dialyzed through cellophane and collodion membranes (29). In electrophoresis at pH 6.54 it moves towards the anode at a rate of $5.4 \times 10^{-5}$ cm/sec/V (29). Bergström prepared a pharmacologically active methyl ester of prostaglandin. Acetylation of the ester gave an inactive product, which became active again on saponification (14). These findings together with the solubility properties of prostaglandin and its instability to bromine suggest an hydroxy acid with an unsaturated long carbon chain.

*2. Pharmacological actions. Smooth muscle preparations.* Prostaglandin stimulates smooth muscle. Purified material acts in minute amounts. The order of activity is similar to that of acetylcholine (14). For biological assay, the action

of prostaglandin on the isolated rabbit jejunum is used. Prostaglandin also contracts intestinal preparations, uterus and bladder from other animals (27, 28, 29, 41). The contraction produced by prostaglandin in the isolated guinea pig ileum proceeds slowly when compared with that produced by histamine (25, 29). The action is followed by a period of increased sensitivity to histamine (90). In this respect prostaglandin resembles other gut-contracting lipid-soluble acids, *e.g.*, unsaturated fatty acids and Darmstoff.

According to Goldblatt (41) the oxytocic action is due to contaminating substances. Nevertheless, it seems reasonable to ascribe to prostaglandin a stimulating action on the uterus, since Euler obtained stimulation with extracts purified by ether extraction at acid pH (29). These extracts should have been free from histamine or other interfering basic substances which could account for the main oxytocic activity of Goldblatt's extracts.

The smooth muscle wall of the tubal ostium in rabbits responds exceptionally in that its tone is lowered by prostaglandin (12). In isolated seminal vesicles of the guinea pig prostaglandin has no stimulating action of its own. However, after a previous stimulation by adrenaline prostaglandin evokes rhythmical contractions (41). Several authors have shown that prostaglandin acts on intestine and uterus *in vivo* (12, see also 31). The action of prostaglandin is not antagonized by atropine (29, 41; but see page 430).

*Blood pressure and blood vessels.* On intravenous injection into cats and rabbits, prostaglandin is hypotensive; even the amount present in 0.05 ml of human seminal fluid is sufficient to cause a fall in arterial blood pressure (27–30, 41).

In contrast to the short-lasting depressor effect of acetylcholine and substance P, that induced by prostaglandin lasts up to one half hour. Sometimes there is no full recovery throughout the whole time of an experiment (27–30, 41). According to Euler the depressor action is due neither to inhibition of the heart nor to peripheral vasodilatation (30). Since the vessels of lungs and liver perfused with defibrinated blood are contracted by prostaglandin, this vasoconstrictor action is assumed to be the cause for the depressor effect. The hypotensive action is not antagonized by atropine. It is obtained in decerebrated animals and in animals anaesthetized with urethane or ether.

*3. Occurrence.* For the study of the distribution of prostaglandin acid-alcohol and ether extracts from various organs were prepared and the gut-stimulating and vasodepressor activities were measured (32). For comparison a standard preparation was used, 0.1 mg of which was defined as one unit. The prostaglandin standard was active on the isolated rabbit jejunum in a dilution of $10^{-6}$ (0.01 unit/ml) (31).

Prostaglandin was found in prostate and vesicular glands and their secretions in humans and sheep (32). Seminal fluid of young men contained 20 to 30 units/ml (31). In sheep prostate glands about 2 to 5 units/g tissue were found (30). Curiously enough extracts of male accessory genital glands from other species did not exhibit prostaglandin-like activity (32). Only traces were found in the ovaries and none in other organs of all species investigated (32). Intestinal extracts exerted some stimulating activity on smooth muscle without correspond-

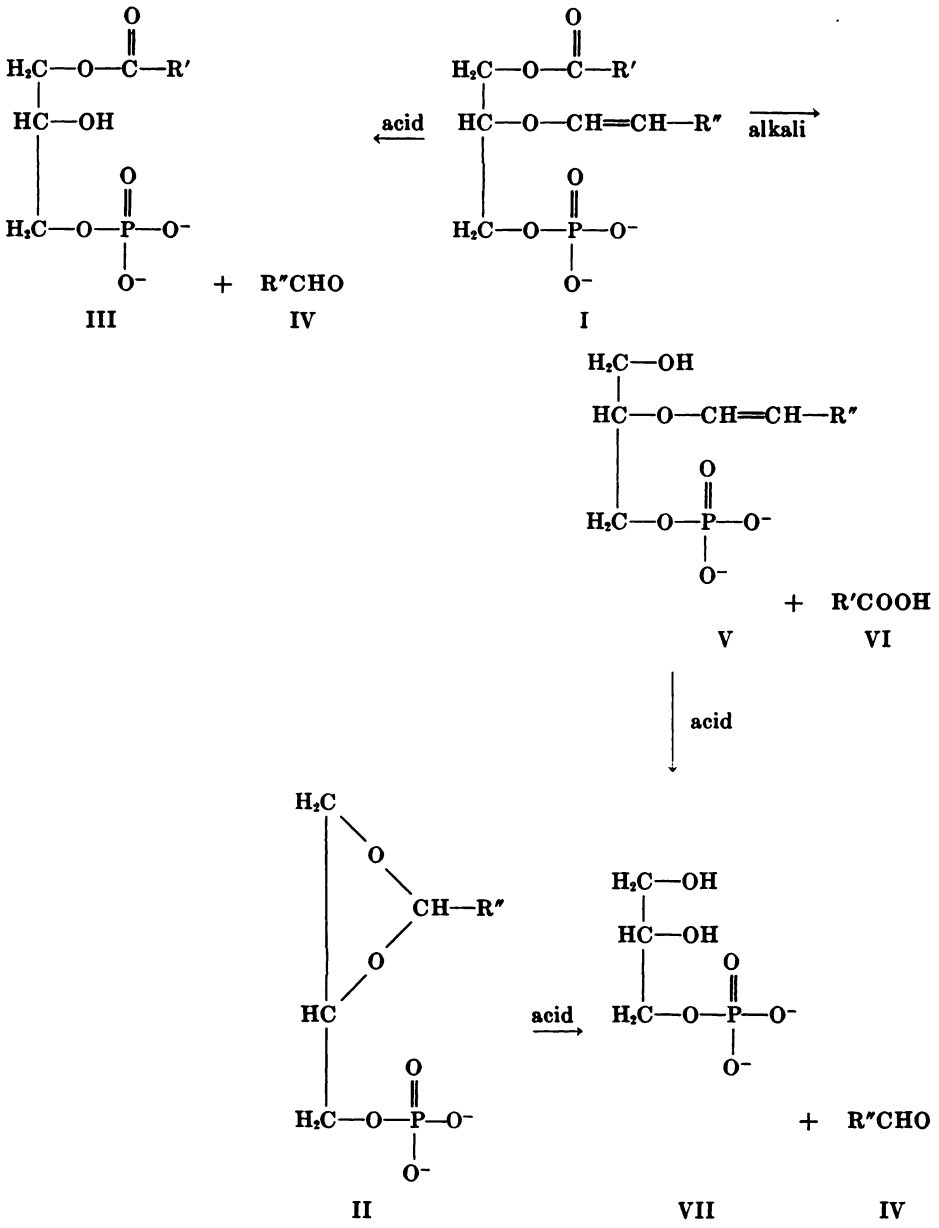
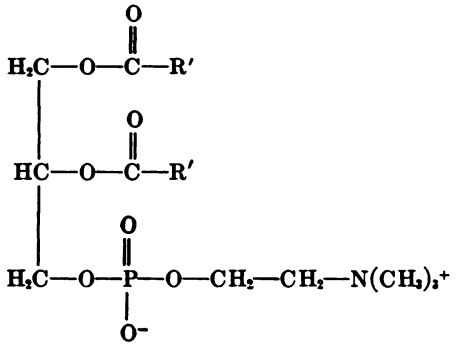



FIG. 1

I = acetalphosphatidic acid, constructed in analogy to the modern structure of acetalphosphatides, suggested by Rapport *et al.* (73a). II = acetalphosphatidic acid, according to Feulgen and Bersin (37a). III and V = phosphatidic acids, derived from I by mild acidic or alkaline hydrolysis, respectively. Both compounds have a "lyso"-structure, *i.e.*, one of the glycerol hydroxy groups is free and one masked by the long carbon chain of a fatty aldehyde or acid, respectively (see also IX and X). IV = fatty aldehyde, and VI = fatty acid, components of phospholipids. VII = α-glycerophosphate. VIII = lecithin. IX = lysolecithin. X = lysophosphatidic acid, corresponding to IX. XI = hydroxy-fatty acid (one R may be H). XII = lactone form of XI. XIII = *cis*-Δ-3-octadecenoic acid (G-acid). XIV = *cis*-Δ-11-octadecenoic acid (*cis*-vaccenic acid). R′ = carbon chain of a fatty acid, varying in different molecules. R″ = carbon chain of a fatty aldehyde, varying in different molecules.

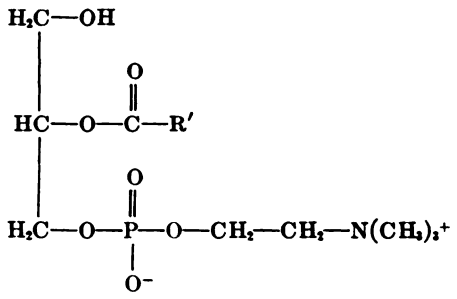
I, II, III, V, X are not to be regarded as precise structures of existing Darmstoff components, but only as principal possibilities.

FIG. 1 (*Continued*)

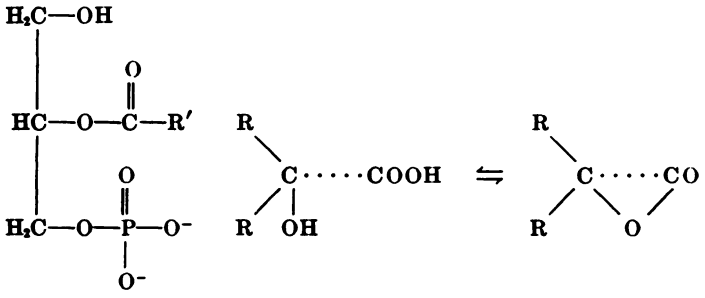


VIII

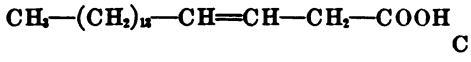
phospholipase A



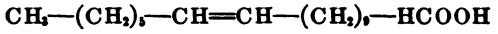
IX



X XI XII



XIII



XIV

ing depressor action; this activity may have been due to Darmstoff. Goldblatt (41) found prostaglandin in human seminal fluid but not in extracts of human prostate. His extracts had not been purified and they contained a smooth muscle-inhibiting principle (adenylic acid?); any prostaglandin effects may therefore have been masked.

*4. Physiological significance.* It has been suggested that prostaglandin promotes ejaculation by inducing peristalsis of the male genital tract (31, 41). The finding

that the amounts of prostaglandin in prostate glands vary with different age groups and are rather low in some cases of infertility (31) would seem to support this assumption. However, Asplund (11) found no correlation between the concentration of prostaglandin in human sperm and fertility; sperm without spermatozoa had the same range of prostaglandin activity as sperm with a normal content of spermatozoa.

Injection of prostaglandin into the uterine or vaginal lumen of rabbits lowered the tone of the tubes whereas the uterine motility was increased (12). This effect was thought to facilitate transport of sperm through the uterus and up the tubes. On the other hand, Barnes [quoted from Asplund (12)] did not see any motor effect of sperm on the rat uterus *in vivo*.

Another possibility as to how prostaglandin could affect impregnation would be by a stimulating effect on the motility of the spermatozoa (11). All these suggestions are at present hypothetical.

*5. Preparation of prostaglandin.* Goldblatt added four volumes of alcohol or acetone to seminal fluid (41). The supernatant obtained from this mixture by centrifugation was evaporated and redissolved in water for assay. Such extracts probably contain more than one pharmacologically active substance. This is illustrated by their oxytocic activity, which is not due to the depressor principle, prostaglandin.

Euler achieved a more efficient purification (28, 29) by extracting the active principle with ether from acidified aqueous solution. This procedure separates prostaglandin from amines and peptides. By adding barium hydroxide much inert material is precipitated and removed, leaving the soluble barium salt of prostaglandin in solution, from which it can then be obtained by evaporation and stored in a stable state. Bergström purified such a preparation considerably, using chromatography and countercurrent distribution (14). Details of his procedure have not been published. Eliasson (25) extracted prostaglandin from prostate glands with hot acid-alcohol. The concentrated extract was freed from unsaponified matter by extraction with ether at alkaline pH. The active principle was then extracted by ether at acid pH together with fatty acids; it was then separated from the saturated fatty acids by the lead acetate method (48).

VESIGLANDIN. Extracts of the vesicular and prostate glands of rhesus monkeys produce hypotension as does prostaglandin. However, these extracts do not have an effect on plain muscle preparations, *e.g.*, intestine and uterus, comparable to that of prostaglandin. The active principle was called vesiglandin to distinguish it from prostaglandin (29). In solubility pattern and in its behaviour towards electrophoresis, dialysis, and precipitating agents vesiglandin resembles prostaglandin. However, at pH 4 vesiglandin is quickly destroyed by boiling, whereas prostaglandin is stable in this range of pH.

These findings lead to the conclusion that prostaglandin and vesiglandin are different, though related, substances. The possibility that prostaglandin consists of a mixture of a principle with stimulating activity upon smooth muscle and a depressor substance (= vesiglandin), has been considered but rejected on the basis of the different stability towards boiling at acid pH (29).

### *B. Darmstoff*

Tyrode solution or other physiological salt solutions acquire smooth muscle-stimulating activity after a piece of intestine has been placed in them for some time. This action is only in part due to choline which diffuses out of the intestinal tissue, as shown by Weiland (92) and Le Heux (66). At least one other compound contributes to the stimulating effect of intestinal bath fluids on the gut, since an effect can be obtained in the presence of atropine (80). An active principle, called Darmstoff, was extracted from such bath fluids, and later from intestinal tissue itself. Its properties were those of a lipid-soluble acid and it has recently been characterized as a mixture of closely related and as yet unseparated acidic phospholipids. A review on Darmstoff has recently been given elsewhere (89).

*1. Physical and chemical properties. Stability.* Boiling Darmstoff in weakly alkaline or acidic aqueous solution for several minutes results in some loss of activity. The loss varies with different extracts. This is probably due to the non-uniform nature of Darmstoff. Active extracts obtained by boiling horse intestine in 0.2 N NaOH were considerably more stable to boiling in 1 N NaOH than to boiling in 0.1 N HCl (83, 88), suggesting an acetal linkage[2]. Darmstoff is destroyed by permanganate, indicating an unsaturated compound (83).

*Solubility.* In the free acid state Darmstoff is only slightly soluble in water, but dissolves in alcohols, ketones, esters, ether, benzene and chloroform (80, 83). It forms water-soluble salts with alkali ions. The partition of Darmstoff between water and organic solvents such as ether or methylethylketone is dependent on the pH of the partition system, in a way that clearly demonstrates an acidic nature (83). From concentrated alcoholic solution Darmstoff is precipitated by barium ions (88), but a considerable proportion of activity remains in the supernatant even with an excess of $Ba^{++}$. This may be due either to a certain solubility of the Ba-salt or to separation of the Darmstoff into two components, the Ba-salt of one being more soluble than that of the other. Lead acetate precipitates Darmstoff from aqueous solution. The lead salt dissolves in aqueous butanol (83).

*Countercurrent distribution.* In a system consisting of the two phases of M/15 phosphate buffer pH 5.6, methanol, *n*-butanol, benzene (5:5:3:6 vol) Darmstoff accumulates in the middle tubes according to a partition coefficient of about 1 (83). The same result is obtained when a solvent system of M/15 phosphate buffer pH 6.2 and methylethylketone is used. In both instances, elevation of the pH results in a shift of the Darmstoff maximum towards the aqueous side.

*Paper chromatography.* When paper chromatograms of Darmstoff are developed with the upper phase of a mixture of methylethylketone-diethylamine-water

[2] Acetals are formed by combination of an aldehyde with two alcoholic groups: $2ROH + RCHO \rightarrow \begin{matrix} RO \\ \\ RO \end{matrix}\!\!>\!CHR + H_2O$. Acetals are easily split into their components by acid. They are resistant to alkali. This stability pattern is peculiar to acetals and unsaturated ethers (see Formulae I and V) which on acid hydrolysis give rise to the formation of one alcoholic and one aldehyde group.

(60:3:20 vol) the biological activity is found in a section of the paper strip corresponding to an $R_f$-value of 0.4–0.45 (83, 88). When the chromatograms are developed with *n*-propanol-concentrated ammonia-$H_2O$ (6:3:1 vol) the $R_f$-value is 0.75–0.9, sometimes separating into two spots which are placed close to each other (88). In water-saturated *n*-butanol Darmstoff causes heavy streaking (88); in chloroform-lutidine-acetic acid (4:4:1 vol) it separates into two areas of biological activity, one remaining at the origin, the other having an $R_f$-value of 0.85–0.9 (90).

*Colour reactions on paper chromatograms.* Provided sufficient material has been applied to the paper the regions which contain the biological activity show a blue spot when stained with the phosphate reagent of Hanes and Isherwood (45). A weak ninhydrin reaction is obtained in the same places, except in chromatograms of the precipitated Ba-salt of Darmstoff (88) and in the upper spot ($R_f$ 0.85–0.9) of chloroform-lutidine-acetic acid chromatograms (90).

The phosphate-positive areas which correspond to the regions of biological activity give a purple colour when treated with acetic acid and subsequently with fuchsin-sulphurous acid, indicating an aldehyde group (88). This was shown in chromatograms developed with propanol-$NH_3$ or with the methylethylketone mixture.

*Other properties.* In paper electrophoresis at pH 6.65 Darmstoff moves towards the anode (83). At pH 2.88 and 2.0 no movement is seen. This indicates a weak acid which in the unionized state has but slight solubility in aqueous medium.

Darmstoff is adsorbed from anhydrous butanol solution by alumina. Elution can be effected by aqueous butanol or aqueous acetone (83). Anion exchange resins also adsorb Darmstoff (83).

*Chemical nature.* The solubility of Darmstoff in organic solvents, its acidic nature and its phosphorus content suggested an acidic phospholipid, *e.g.*, a phosphatidic acid. Further, the stability to alkali and the sensitivity to acid, together with a positive aldehyde reaction after hydrolysis, indicated an acetal linkage. The conclusion that the active principle might be an acetalphosphatidic acid (II or V) was supported by the following findings. Treatment with acetic acid resulted in the formation of a free lipid-soluble aldehyde (IV) and of an organic phosphorus compound which was indistinguishable from $\alpha$-glycerophosphate (VII) in paper chromatograms (88). Both compounds are products expected to be formed from acetalphosphatidic acid[1]. The treatment with acid was accompanied by a loss of biological activity, indicating that the destroyed acetalphosphatidic acid was an active principle. However, after the treatment with acid there still remained some gut-stimulating activity which has been attributed to other phospholipids which are resistant to the mild acidic hydrolysis (88). The nature of these more stable acidic phospholipids is unknown. Sphingolipids are known to

[1] This conclusion was originally based on the classical structure of acetalphosphatidic acid (II); it fits also the modern formula (V) which represents an acetalphosphatidic acid derived from the genuine compound (I) after treatment with alkali. The Darmstoff extract used in the experiments mentioned above, in fact, had been boiled with 0.2 N NaOH. The genuine Darmstoff compound may, therefore, correspond to (I).

be rather stable to alkali and acid. However, in hydrolysates obtained by refluxing Darmstoff with 6N HCl or with methanolic $H_2SO_4$ no sphingosine was found (90).

To consider Darmstoff as a mixture of acidic phospholipids is consistent with known properties of phosphatidic acids as regards solubility of Ba-salts and movement in paper chromatograms developed with *n*-butanol (52) or chloroform-lutidine-acetic acid (76). The ninhydrin-positive component which did not precipitate with $Ba^{++}$ and which in chloroform-lutidine-acetic acid did not move on paper, may be an unknown amine-containing acidic phospholipid or a complex of a phosphatidic acid with an amine.

Some alkali-treated Darmstoff extracts showed a haemolytic action. They also inhibited the contractility of isolated strips of guinea pig ileum. This may indicate the presence of lysophosphatidic acid (see below).

*2. Pharmacological actions. Smooth muscle.* Darmstoff increases the tone of the longitudinal muscle of isolated preparations of the small intestine of dog, cat, rabbit, rat, mouse and guinea pig (1, 59, 80, 84) and may evoke rhythmic activity in the guinea pig ileum and frog rectum where such activity is often absent (59, 80, 89). Usually the frequency of the rhythmical contractions remains unchanged, but in the frog rectum the frequency increases considerably with only a slight rise in tone (80). The isolated rabbit duodenum, which has been widely used for the biological assay of Darmstoff, is particularly sensitive and reacts to purified Darmstoff preparations in concentrations of less than $10^{-8}$ (83). When the lumen of the isolated guinea pig ileum is filled with fluid at subthreshold pressure, peristaltic contractions are elicited on the application of Darmstoff (84). On intravenous injection Darmstoff stimulates rabbit intestine *in vivo* (84).

The isolated uterus of rabbit, rat and guinea pig and the isolated urinary bladder of frog, rat and rabbit are stimulated by Darmstoff (59).

The stimulating actions of Darmstoff on smooth muscle can be obtained in the presence of atropine [up to $3 \times 10^{-7}$ in isolated organs (80) and 1 mg/kg in whole rabbits (84)]. Nevertheless, these responses are not entirely resistant to atropine in all preparations. In the intestine of the guinea pig, but not of the rabbit, the stimulating action of Darmstoff is reduced in the presence of atropine $10^{-8}$ and even abolished when small doses of Darmstoff are used (89). Antihistaminic, sympatholytic and ganglion-blocking agents do not inhibit the responses to Darmstoff (84). Botulinum toxin is an effective antagonist (1). The contractions elicited by Darmstoff in the isolated rabbit duodenum are strongly augmented by the anticholinesterase compound BW 284C51 [1,5-bis-(*p*-allyldimethyl- ammoniumphenyl)pentan-3-one dibromide] (1, 2). Another kind of synergism is seen in the guinea pig ileum. The application of Darmstoff is often followed by an increased sensitivity of the ileum to histamine and acetylcholine, for as long as ten minutes (89).

Recently, an unexpected action of some Darmstoff preparations was observed. These extracts stimulated the rabbit intestine, but did not contract the guinea pig ileum, in fact, they inhibited the response of subsequently applied histamine and acetylcholine (89). This inhibiting action was similar to that of lysolecithin

(IX) (33, 44, 74, 86) and it was assumed that it might be produced by lysophosphatidic acid. (*e.g.*, III, V, X). This was supported by the finding that the extract with inhibitory activity had a haemolytic action. The excitability of the rabbit duodenum is not impaired by lysolecithin; this again is in accordance with the action of the extracts presumably containing lysophosphatidic acid. The inhibiting action was obtained with Darmstoff preparations which had been treated with NaOH, but also with some other samples. Assumptions as to the origin of the inhibiting compounds center on acetalphosphatidic acids (I), which might be partly split by alkali or acid, in both cases yielding lysolecithin-like structures (III, V). However, no conclusive results have as yet been obtained.

*Mechanism of action.* Darmstoff does not act directly on the smooth muscle fibers. This is apparent from the following findings. 1) Darmstoff does not act on intestinal preparations which have been stored in the cold for some days, although these peparations still respond to acetylcholine (84). 2) The action of Darmstoff is inhibited by botulinum toxin and potentiated by the anti-cholinesterase BW 284C51 (1). 3) The action on the rabbit duodenum is not antagonized by atropine, but in the guinea pig ileum there is an antagonistic effect of atropine (89). The second and third finding indicate a nicotine-like action (26) which is assumed to be neuronal and cholinergic. The evidence is discussed in a review of Ambache (2). However, in the author's opinion, other explanations seem also worth considering, which explanations would be apt to circumvent the discrepancies arising from the postulation of atropine-resistant cholinergic actions. There might be two different mechanisms of action: a non-cholinergic action which would predominate in the rabbit intestine, and a cholinergic one predominant in the guinea pig ileum which would not necessarily be neuronal. The acetylcholine released might also be derived from non-nervous structures. These problems are discussed in extension elsewhere (89).

*Other actions.* Darmstoff does not contract the *rectus abdominis* of the frog, but potentiates the effect of acetylcholine on this preparation (80, 89). The increased sensitivity often persists after the Darmstoff in the bath fluid has been washed out.

Intravenous injections of Darmstoff in doses which cause strong contractions of the intestine *in situ* have no effect on the arterial blood pressure of rabbits anaesthetized with urethane (82, 84).

*3. Occurrence.* Darmstoff diffuses out of intestinal tissue into the surrounding bath fluid (80, 81). The concentration of freely diffusible Darmstoff in rabbit intestine was found to range from 2 to 10 units/g tissue (81). On extraction with organic solvents from homogenized intestinal tissue 140 to 400 units/g tissue were obtained (90). Considerably higher yields—up to 5000 units/g—were obtained by boiling the intestine in two volumes of 0.2 N NaOH (88). From these results it appears that most of the Darmstoff is bound and becomes extractable only after disintegration of the tissue.

It is important to note that the figures given are tentative. The high values obtained by boiling with alkali refer to crude extracts which may contain other lipid-soluble acids, *e.g.*, "fraction X", discussed below. On purification considerable losses were encountered (83). It is also possible that in the alkali-boiled extracts

part of the Darmstoff is not preformed, but is produced during extraction. On the other hand, in extracts obtained by organic solvents part of the Darmstoff may be bound to cationic compounds in such a way as to alter the physico-chemical properties with the result that the bound Darmstoff is lost during the fractionation processes. These are problems now under investigation.

Little is known about the occurrence of Darmstoff in tissues other than the intestine. In dialysates of isolated pieces of skeletal muscle from rabbits no Darmstoff was detected. On the other hand, extraction of homogenized skeletal muscle with acetone and alcohol-ether mixture (3:1) led to the detection of a smooth muscle-stimulating principle which in paper chromatography and countercurrent distribution behaved like Darmstoff. The yield was about 50 units/g muscle (90).

*4. Physiological significance.* The occurrence and action of Darmstoff suggest that it plays a physiological role in the motor activity of the gut. When the rhythmical contractions of the frog rectum disappear after several changes of the bath fluid they can be evoked again by applying the Darmstoff recovered from the bath fluids (80). The concentration of Darmstoff present in a freely diffusible state in rabbit intestine *in situ* is greater than the threshold concentration necessary for stimulation (81). It is an attractive idea to assume that Darmstoff is the postganglionic transmitter for atropine-resistant vagal impulses. The indirect mode of action of Darmstoff speaks against such an assumption, as pointed out by Ambache (1). However, it does not exclude the possibility that on vagal stimulation processes are initiated or accelerated by which Darmstoff is released. That a substance, resistant to atropine, which contracts the gut appears in the bath fluid of frog stomach preparations after vagal stimulation is in favour of this possibility (80). The substance released has not yet been characterized.

*5. Preparation of Darmstoff.* The first extracts were obtained by drying dialysates of frog intestine *in vacuo* and extracting the residue with chloroform (80). The resulting extract is free from gut-stimulating compounds other than Darmstoff. The yield of active material is low. Boiling horse intestine in water is a more efficient method for obtaining larger quantities. Such boiled extracts can be concentrated and extracted with butanol. The butanol extract can be purified further by countercurrent distribution and paper chromatography on large sheets (83). The tedious procedure of chopping the tissue before extraction can be circumvented by boiling the intestine in 0.2 N NaOH which results in dissolution of the tissue. By acidifying the solution with HCl, Darmstoff is precipitated with the proteins and is extracted from the precipitate by means of butanol, leaving the proteins behind. Further purification is effected by countercurrent distribution (88).

With respect to the chemical nature of Darmstoff the two large scale methods mentioned above appear rather rigorous and likely to destroy or alter some of the active principles. Pure solvent extraction would ensure that no changes occur in the chemical structure. So far, only preliminary results have been obtained using acetone and alcohol-ether for extraction from homogenized tissue (90).

*Standard.* A sample of Darmstoff from dialysates of frog intestine was mixed

with NaCl and used as a dry standard preparation. One unit was defined as 10 μg of this preparation (81). This amount in one ml of Tyrode solution was about threshold concentration for preparation of rabbit duodenum.

### *C. Irin*

On stimulation of the trigeminal nerve in rabbits the *sphincter iridis* is contracted, a response characterized by a long duration and by resistance to atropine. This suggested to Ambache that the mediator for the antidromic trigeminal stimulation—if there was any—differs from acetylcholine (5). Ambache extracted an active principle—"irin"—from rabbit iris which contracted iris muscle *in vivo* in fully atropinized, atropinesterase-free rabbits (3, 5).

*1. Physical and chemical properties. Solubility.* Irin is soluble in water, in aqueous or pure acetone, in ether and chloroform (5). Its distribution between water and chloroform and between water and ether depends on the pH. At alkaline pH irin is found in the aqueous phase whereas at acidic pH it moves into the organic layer. This fact indicates a lipid-soluble acid. In paper chromatograms irin moves readily with organic solvents. Its $R_f$-value in *n*-butanol-acetic acid is 0.91 to 0.98 (5); in methylethylketone-diethylamine the $R_f$ is 0.9 (8).

*Stability.* Originally, crude extracts from iris (pH 6.1 to 7.0) were placed for 2 to 3 min in a boiling water bath as a routine procedure. Later experiments showed that this results in a loss of activity and, in fact, irin is thermolabile (6). Chymotrypsin and alkaline phosphatase do not destroy it (5, 6). By treatment with permanganate or IBr the activity is lost, indicating an unsaturated compound (7).

*Other properties.* In electrophoresis on filter paper irin moves towards the anode provided the pH of the supporting buffer is above neutral. At pH 6.7 to 7.0 there is only slight anodal movement and at lower pH there seems to be no movement at all (5). This behaviour of irin suggests a weak acid which may be adsorbed or is not sufficiently soluble in the free unionized state.

Purified irin spread on paper in spots does not react to ninhydrin and does not stain for $NH_2$-, aldehyde or acetal groups (8). From reactions with phenyl*iso*cyanate, with *sym* diphenylcarbazide and from the infrared spectrum it is suggested that irin is an unsaturated lactonizing hydroxy-fatty acid (XI, XII) (7). The fact that various lactones interact with irin, either by potentiation or by inhibition, suggests that the active form is the lactone (7).

*2. Pharmacological actions.* Irin is a smooth muscle stimulant. It contracts the sphincter pupillae and isolated preparations of intestine from various species.

*Iris.* The action of irin on the iris muscle is of physiological interest. Isolated ox sphincter preparations are strongly contracted by iris extracts corresponding to 1 mg of fresh iris tissue (0.3–1 μg of purified irin) per ml organ bath (5). The contraction is long lasting and subsides only slowly after the bath fluid is changed. Neither atropine ($10^{-8}$ to $10^{-6}$) nor mepyramine ($10^{-5}$) is antagonistic. When irin extracts are injected into the anterior chamber of the eye in cats or rabbits a long lasting miosis develops (4, 5). In a cat which had been given 3 mg/kg atropine intravenously, an injection into the anterior chamber of irin in a dose

corresponding to 2.9 mg of iris constricted the pupil for about an hour to less than 1.5 mm (5).

*Other smooth muscle organs.* Isolated preparations of rat intestine and hamster colon are stimulated by small amounts of irin. Rabbit and guinea pig ileum are less sensitive. The action of irin is not abolished by atropine $10^{-6}$; in the guinea pig ileum it is, however, sometimes depressed as is the action of histamine. On the other hand, occasionally even an augmentation by atropine of irin-induced contractions was observed (5).

Most investigations and routine assays of irin were carried out with the diagonally striated portion of the rat colon. This organ is particularly sensitive, responding to purified irin in a concentration of $10^{-6}$ with a marked contraction (5). Since the rat colon is insensitive to histamine and bradykinin and since neither atropine nor lysergic acid diethylamide ($2 \times 10^{-7}$) antagonizes the action of irin, known substances such as acetylcholine, 5-hydroxytryptamine, histamine and bradykinin can be excluded as possible active principles. Atropine enhances the response of the rat colon to irin. Recently, the hamster colon has superseded the rat colon for the bioassay of irin (6).

Irin does not contract the isolated rat uterus but potentiates its response to bradykinin (5).

*3. Physiological significance.* Irin was found not only in extracts of iris tissue but also in aqueous humor after paracentesis (4) and, occasionally, traces of it appear after mechanical stimulation of the trigeminal nerve (8). Both these procedures are followed by prolonged, atropine-resistant, pupillary constriction. On the other hand, in aqueous humor obtained from unstimulated eyes, little or no irin was detected. Thus, irin is apparently liberated on antidromic stimulation of the trigeminal nerve and may well be the chemical transmitter or a local hormone for producing the effect of such stimulation, namely, spasm of the iris.

### *D. Slow reacting substances*

The term "slow reacting substance" (SRS) was first used by Feldberg and Kellaway (36) to describe an unknown smooth muscle-stimulating principle which appeared in the perfusate from guinea pig lung after injection of cobra venom. Slow reacting substances were, thereafter, found in Kellaway's laboratory when cell injury was induced by injection of venoms, bacterial toxins, peptone, trypsin, antigen, or volatile anaesthetics. The typical response to SRS was a rather slowly proceeding contraction of the isolated guinea pig ileum, followed by a slow relaxation, after the bath fluid was changed.

SRS was not assumed to be identical in all cases, but the term simply served to characterize the liberated substances by their pharmacological action, since any indications of their chemical nature were lacking. Later, the term SRS or SCS (slowly contracting substance) has been used by other investigators who found similarly acting substances under various physiological or pathological conditions. Naturally, the reader may be inclined to think of one and the same substance or at least of one group of closely related substances. However, two entirely different groups of slow reacting substances have been established; one

of these consists of polypeptides, the other one of lipid-soluble acids. To the group of polypeptides belongs bradykinin formed when (75) trypsin acts upon serum globulins (bradykinin means "slowly acting"). Bradykinin is also formed by the action of other proteolytic enzymes such as plasmin (67), or by the enzymes present in snake venoms or in tissues (49, 75) and bradykinin-like substances appear during anaphylactic shock in dogs (13) or during the antigen-antibody reaction in the isolated guinea pig ileum (85). This group of slow reacting substances will not be considered in this review. It is interesting that the processes that lead to the formation of polypeptides, namely, the antigen-antibody reaction and the action of snake venoms, also produce lipid-soluble acids which in some respects have similar pharmacological properties.

The acidic slow reacting substances which have been roughly identified are unsaturated fatty acids; for this reason, other unsaturated fatty acids have been included in this chapter though they have not been termed SRS. On the other hand, one SRS (SRS-A) is probably not an unsaturated fatty acid.

G-ACID. The well-known property of serum or plasma to stimulate smooth muscle has to be attributed to several substances. One is the "Spätgift" of Freund (38), later called serotonin and identified as 5-hydroxytryptamine (73). Another one is a bradykinin-like peptide or group of peptides (10, 68, 77). Gabr (39) found a slow contracting substance in the "G2-fraction" (56) of human plasma; this substance had the properties of an unsaturated fatty acid and it was called G-acid. G-Acid appears to be preformed in plasma or only loosely bound (lipoprotein complex?) (47) so that it is liberated by extraction with alcohol.

*1. Physical and chemical properties.* G-Acid has the properties of a straight-chain unsaturated monocarboxylic acid. Its neutral equivalent is 283, its iodine number 75. Hydroxyl or ketogroups are apparently absent. The free acid is readily soluble in ether and other solvents, but insoluble in water. Its Ca salt is also insoluble in water. On oxidation with permanganate only one dicarboxylic acid was obtained, which by paper chromatography was identified as malonic acid. Another split product was a monocarboxylic acid, which by paper chromatography was indistinguishable from pentadecanoic acid. From this it was concluded that G-acid is Δ-3-octadecenoic acid (XIII), an unsaturated fatty acid hitherto not observed in living matter. Several chemical tests seem to rule out major contamination with other fatty acids.

*2. Pharmacological actions. Isolated intestine.* For the study of the actions of G-acid on smooth muscle the isolated guinea pig jejunum has mainly been used. In a bath of a capacity of 10 ml this organ is contracted by 20 to 50 μg of G-acid (39). Fifty mg of the G2-fraction of human plasma give a response of similar strength. The contraction starts after a latency of 20 to 30 sec and then proceeds slowly, reaching its maximum after 1 to 2 min. Relaxation after washing out is slow. From Gabr's figures the substance appears to act without tachyphylaxis. The rabbit jejunum, too, is contracted by G-acid and whole G2-fraction of plasma. With this preparation the contraction is rather quick, though some time passes before its onset. The isolated rat colon does not respond to G-acid.

The action of G-acid is not antagonized by atropine nor by the antihistaminic antazoline.

*Coronary arteries.* The G2-fraction of human plasma has a constrictor effect on the coronary arteries of the perfused isolated rabbit heart. Purified G-acid has a similar effect, which, however, is only 7.5 times that of whole G2-fraction. In this connection it is important to note that the gut-stimulating activity of purified G-acid is about 1000 times greater than that of whole G2-fraction. Thus the constrictor effect may be mainly or wholly due to another substance.

*Haemolysis.* G-Acid is markedly haemolytic. This effect is absent in whole G2-fraction of plasma. It is assumed to be masked by plasma proteins.

*3. Preparation of G-acid.* The active principle was first separated from proteins by adding alcohol to an aqueous suspension of the G2-fraction. A clear filtrate was obtained which was concentrated and extracted with acetone. The material extracted by the acetone was adsorbed on an alumina column from a solution of 10% acetone in methanol. Elution was achieved by 50% aqueous methanol. The substance was then precipitated from aqueous solution by acetic acid and crystallized from ether.

HAEMOLYTIC ACID (*cis*-VACCENIC ACID). It is well known that haemolytic substances occur in the blood and tissues which might have a bearing on physiological destruction of aged erythrocytes (for references see 63). Laser and Friedmann (65) were able to obtain a crystalline compound from human plasma which was strongly haemolytic in high dilutions ($10^{-5}$). Contrary to the ideas of most of the earlier workers, the substance was not lysolecithin, as it was entirely free of nitrogen and phosphorus.

*1. Chemical and physical properties.* The haemolytic agent was characterized by Laser as an unsaturated monocarboxylic acid; the analytical data fitted a C18 fatty acid (62, 63). Morton and Todd (71) identified this haemolytic substance as *cis*-Δ-11-octadecenoic acid (*cis*-vaccenic acid) (XIV). Evidence for this structure was given by absorption spectra, oxidative fission of the molecule at the site of the double bond and determination of the resulting split products. Further, synthetic *cis*-Δ-11-octadecenoic acid had the same melting point and there was no depression when the synthetic compound was mixed with the naturally occurring product.

The sample used for identification did not originate from blood but from brain, which is particularly rich in this compound (63).

*2. Pharmacological effects. Haemolysis.* It is a well-known property of unsaturated fatty acids to cause haemolysis. The haemolytic acid of Laser, however, is more potent in this respect than other fatty acids, *e.g.*, oleic acid (71). The haemolytic effect is inhibited by a number of normal constituents of the body, such as serum albumin, cholesterol, lecithin and calcium ions (63). Of special interest is the apparent connection between the haemolytic acid and antimalarial drugs, which have been shown almost specifically to inhibit *in vitro* haemolysis by the acid (61, 62). Thus, under given experimental conditions, quinine in a dilution of $10^{-4}$ prolonged the time necessary for complete haemolysis from 1 min to

20 to 30 min (61). Derivatives of antimalarials which are ineffective against the disease failed to antagonize the haemolytic action of *cis*-vaccenic acid.

*Smooth muscle.* The isolated guinea pig ileum is contracted by the haemolytic acid (39, 64) as it is by other unsaturated fatty acids.

*3. Occurrence.* The haemolytic acid was first extracted from plasma (65). It is, however, widely distributed in the body, with the highest content found in brain (63, 71). That the haemolytic substance extracted from different sources is identical, is based 1) on the similar method of extraction and purification—preparation of an ether-soluble lead salt—and 2) on equal haemolytic activity, on a weight basis, of the compound obtained from the various sources (63). In erythrocytes the substance seems to be bound to the stroma (63). In malaria-infected red cells of monkeys it is present in higher amounts than in normal controls (62).

*4. Physiological significance.* As yet there appears to be no significant role for the *cis*-vaccenic acid found in brain (63). However, its occurrence in red cells led to the assumption that it might effect the elimination of aged erythrocytes. It has been assumed that the acid is inactive when bound to the stroma, and is slowly released from the stroma into the cytoplasm of the red corpuscle, where it might eventually reach a concentration high enough to cause disruption of the cell (63).

As malaria-infected cells contain greater amounts than normal cells, it is assumed that the haemolytic acid is produced by metabolic activity of the plasmodia, causing one of the main phenomena of malaria, namely an increased rate of red cell destruction (61, 62). The production and action of the haemolytic acid is thought to be in some way essential for the life of the plasmodia. Treatment with antimalarial drugs—which inhibit the haemolytic action of *cis*-vaccenic acid—would then be beneficial by interfering with the haemolytic action (61, 65). This is supported by the parallelism between antimalarial and antihaemolytic activity of drugs which is apparent only when the latter action is tested against *cis*-vaccenic acid, and not when other haemolytic agents, such as lysolecithin, or bile acids, are used (61).

SRS-C.[4] When egg yolk is incubated with cobra venom a slow reacting substance (SRS-C) is formed. This was first observed by Feldberg *et al.* (33) during investigations on the mode of action of cobra venom-poisoning. The slow contraction produced in the isolated guinea pig ileum by SRS-C-containing envenomed egg yolk is disturbed and even prevented by interaction of lysolecithin which is formed concomitantly. Only after chemical separation of the two principles can the action of SRS-C be clearly observed.

*1. Physical and chemical properties.* Feldberg *et al.* (33) showed that SRS-C is soluble in acetone, alcohol and ether. By this finding a polypeptide structure was excluded. In view of the fact that other venoms, *e.g.*, that of *Bothrops jararaca*,

[4] This abbreviation has not been used in the literature, before. The term is suggested to distinguish this substance from other slowly acting substances, following a nomenclature of Brocklehurst, who called the SRS appearing in anaphylactic lung SRS-A.

form slowly contracting polypeptides (bradykinin) from suitable substrates the apparently different structure of SRS-C was rather puzzling. Its lipid-solubility was further demonstrated by the fact that it dissolved in benzene and petroleum ether and moved with the solvent front in paper chromatograms developed with *n*-butanol (86). When partitioned between water and ether SRS-C behaved like an acid, moving into the ether phase at acidic pH, but into the aqueous phase at alkaline pH of the solvent system (86).

The free acid was not soluble in water, but dissolved on addition of NaOH. It formed a lead salt which was insoluble in water and soluble in alcohol and ether (86). These are properties of unsaturated fatty acids.

*Formation of SRS-C.* Cobra venom contains phospholipase A, which splits lecithin (VIII) into lysolecithin (IX) and free fatty acid (VI). As SRS-C had the properties of an unsaturated fatty acid it was assumed that it consisted of the fatty acids which are split from egg lecithin by the venom. This was proved by showing that SRS-C was formed on incubation of pure lecithin with cobra venom in ether solution and that it was present in the unsaturated fatty acid fraction of the reaction mixture (86). SRS-C is also formed by alkaline hydrolysis of lecithin (86).

A substance which has the same pharmacological properties as SRS-C occurs in perfusates of isolated perfused tissues after injection of cobra venom (36). The perfusates also contain lysolecithin, indicating an action of the phospholipase A of the venom on tissue lecithin. Accordingly one would expect the liberation of unsaturated fatty acids from the attacked lecithin and assume that the slow reacting substance appearing in the perfusate is identical with SRS-C. This highly probable interpretation has, however, not yet been proved by experiment.

*Nature of SRS-C.* The physico-chemical properties and conditions of formation provide strong evidence that SRS-C is an unsaturated fatty acid derived from lecithin. Lecithin is not uniform in its fatty acid constituents so that the exact nature of SRS-C is still open to question. It is not oleic acid (33, 86). Linoleic acid may contribute to its effect but the smooth muscle-stimulating effect of linoleic acid does not seem to be sufficiently strong to account for the main activity of SRS-C (86). On the other hand, Gabr (39) found linoleic acid to be a highly active substance.

*2. Pharmacological actions. Isolated intestine.* Most studies have been made on the isolated guinea pig ileum. This organ is contracted by SRS-C after a latency which may take several minutes (33, 86). Maximum contraction is reached slowly. The action is followed by characteristic after-effects. Firstly, relaxation after change of the bath fluid is slow; and secondly, the sensitivity of the preparation to histamine and acetylcholine is increased (33, 86). Contractions elicited by histamine may reach twice the height of those before application of SRS-C. This state of hypersensitivity gradually subsides within 5 to 20 min.

The action of SRS-C occurs in the presence of atropine $10^{-8}$ (86) but it is not known whether atropine has an antagonistic effect. SRS-C also contracts isolated rabbit duodenum preparations (90).

*Other effects.* SRS-C has a pronounced haemolytic action which is not due to

contamination with lysolecithin, as was shown by paper chromatography (86). However, when lecithin is split by cobra venom the main haemolytic activity results from lysolecithin, one of the products of hydrolysis; the other split product is the free fatty acid, SRS-C.

On intravenous injection into rabbits SRS-C has a pronounced hypotensive effect (90) similar to that of cobra venom when injected into cats or dogs (33–35). This action may lead to circulatory collapse and death.

*3. Pathological significance.* The formation of SRS-C in egg yolk is without any physiological significance. If we assume that it can be similarly formed by the action of venoms containing phospholipase A on tissue lecithin it might contribute to the symptoms of venom poisoning, *e.g.*, the lowering of blood pressure. Phospholipase A is present also in animal tissues and may become active in an uncontrolled manner by tissue damage.

It must be borne in mind, however, that lysolecithin, which is formed concomitantly with the fatty acids from lecithin, has similar circulatory effects (33, 90). Further, the toxicity of phospholipase A has been found to be small when compared with other fractions of snake venoms (42, 43).

FRACTION X. Extracts of intestinal tissue obtained by methods used to prepare Darmstoff contain a smooth muscle-stimulating principle in addition to Darmstoff (83). This substance, provisionally called "fraction X," is also a lipid-soluble acid as shown by its behaviour in distribution between water and organic solvents (90). The free acid is scarcely soluble in water but readily soluble in alcohols, ketones, benzene and petroleum ether. By countercurrent distribution in a solvent system consisting of the two phases of M/15 phosphate buffer pH 5.6-methanol-butanol-benzene (5:5:3:6 vol) fraction X separates from Darmstoff showing a partition coefficient still more in favour of the organic phase (83).

Like Darmstoff, fraction X stimulates isolated rabbit jejunum and guinea pig ileum preparations. In the rabbit jejunum the action is atropine-resistant; the question of an interaction of atropine in the guinea pig ileum has not been investigated. The blood pressure of anaesthetized rabbits is lowered by fraction X.

Fraction X may well be an unsaturated fatty acid but no experimental evidence has as yet been given.

SRS-A. It is well established that the antibody-antigen reaction liberates histamine, and that the pharmacological actions of histamine contribute to the symptoms of anaphylactic shock and allergy. However, the question has arisen whether these events can be explained by histamine liberation alone. The failure of antihistaminics to prevent certain symptoms has led to the postulation of special actions of "intrinsic" histamine and, on the other hand, even to the negation of the significance of humoral agents in anaphylaxis. Experiments of Kellaway and Trethewie (57) on perfused sensitized guinea pig lungs had, however, suggested the participation of other substances in symptoms of anaphylaxis. These authors observed that after an anaphylactic shock there appeared in the venous effluent of the lungs not only histamine but also another substance with

smooth muscle-stimulating properties. When assayed on an isolated strip of guinea pig ileum the effluent caused a contraction more prolonged than that produced by pure histamine. After the bath fluid was changed the ileum relaxed only slowly, an effect likewise different from that of histamine.

Brocklehurst (19, 20, 21) investigated the properties of the slowly contracting principle which he called SRS-A (slow reacting substance in anaphylaxis). Its action was not due to 5-hydroxytryptamine, bradykinin or histamine. Unlike 5-hydroxytryptamine it contracted isolated human bronchioli, and unlike bradykinin it was not destroyed by proteolytic enzymes. As its action persisted in the presence of mepyramine it was not histamine. By an adsorption technique Brocklehurst succeeded in separating SRS-A from the accompanying histamine. The effluent collected from perfused sensitized guinea pig lungs after injection of the antigen was passed over activated charcoal which adsorbed SRS-A. Elution was achieved by 8% *n*-butanol in water. Using samples thus purified it was shown that SRS-A produces a slowly proceeding contraction of the guinea pig ileum, followed by delayed relaxation after the bath is changed. Contractions produced subsequently by standard doses of histamine were potentiated and the sensitivity of the gut slowly returned to normal. SRS-A contracts the isolated rabbit duodenum, the hen rectal caecum and human bronchioli. It has no effect on the rat colon, on the uterus of guinea pig and rat and on the bronchioli of cat, dog, rabbit and guinea pig, in doses at least 20 times greater than those effective on the guinea pig ileum. SRS-A does not affect the blood pressure of rabbits or cats.

SRS-A was not detected in perfusates or extracts from sensitized lungs without contact with the antigen. After injection of the antigen it could be extracted from the disintegrated lung tissue by 70% alcohol. This suggests that—unlike histamine—SRS-A is not pre-existent in the sensitized tissue but formed in the course of the antigen-antibody reaction.

A slow reacting substance appears also in the effluent of human asthmatic lung perfused with allergen solution. From a comparison carried out with SRS-A from guinea pig lungs it appears that both principles are identical (20).

In electrophoresis at pH 8, SRS-A moves towards the anode. Further evidence for an acidic nature results from the distribution of the substance between water and ether. At pH 3 some of the active principle is taken up by the ether phase, at pH 8 this returns to the aqueous phase (22). However, the partition is much in favour of the aqueous phase, even at pH 3. Thus, SRS-A seems to be an acidic substance with a certain though not predominant lipid-solubility. It would be interesting to see whether it is present as a relatively hydrophilic complex and would show a greater lipid-solubility after being liberated from the complex (see Section IV). The fact that SRS-A sensitizes tissue to the action of histamine is in accordance with the properties of other lipid-soluble acids.

SRS-A is formed by the action of allergen on human asthmatic lung and it produces bronchospasm. This led Brocklehurst to suggest that this principle may be of significance in human asthma—a view that is supported by the fact that many potent antihistamine drugs have only minor effects in human asthma.

### *E. Substances possibly related to lipid-soluble acids*

The following section deals with substances some properties of which indicate lipid-soluble acids though this is not definitely established. Because of their undetermined nature these compounds are described only briefly.

*1. Smooth muscle-stimulating substance appearing in lymph during reactive hyperaemia.* Carlsten *et al.* (23) observed a substance appearing in canine lymph which contracted the isolated guinea pig ileum and was different from histamine or acetylcholine. This compound was present regularly only in lymph originating from regions the blood supply of which had been restricted shortly before collection of the samples. The following biological properties may indicate that the substance in lymph is a lipidsoluble acid: it produces a delayed slow contraction of guinea pig ileum preparations which slowly subsides after washing out, and subsequent to its action the sensitivity of the ileum to histamine is greatly increased, returning to normal gradually.

*2. Eutocine.* At the time of delivery human uterine muscle tissue and amnionic fluid contain a substance called "eutocine" which stimulates the motor activity of the uterus of rats and guinea pigs (46), producing rhythmical contractions. Intestinal muscle is weakly stimulated. The solubility pattern of eutocine indicates a lipid-like compound.

*3. Rhizostomine.* Extracts of a jelly-fish, *Rhizostoma pulmo*, exhibit smooth muscle-stimulating actions which are due to an unknown highly active principle called "rhizostomine" (17). Rhizostomine is soluble in butanol. Its movement in countercurrent distribution—towards the site of lipid material (17)—and its high $R_f$-value—0.8—in paper chromatograms developed with butanol-morpholine-water (2:3:4 vol) (16) indicate a lipid-like substance. In paper electrophoresis no movement was found from pH 3 to 10. This seemingly neutral behaviour does not necessarily exclude an acidic substance.

*4. Pressor substances in plasma.* Gayer and Sarre (40) found a pressor substance in plasma of hypertensive patients which was different from known amines and angiotonin (hypertensin) or vasopressin. The pressor principle can be extracted from acidified aqueous solution by ether. It is assumed to be an acid of high molecular weight which may have some connection with hypertension although the differences in content in plasma of normal and hypertensive individuals are rather small.

A similar principle with pressor activity was found by Masson, *et al.* (69) in human plasma after incubation at 37°C under sterile conditions. Khairallah (58) found it to be soluble in methanol, ethanol and methanol-chloroform mixtures. By addition of acetone it was precipitated from methanolic solution. In paper chromatograms the region containing the pressor principle gave a positive test for phosphorus and choline and stained with Sudan black, suggesting a phospholipid. The pressor substance might be a salt of an acidic phospholipid; this would explain its insolubility in chloroform, ether and petroleum ether (58).

*5. Sensitizing and smooth muscle-contracting substances in brain and cerebrospinal fluid.* Cerebrospinal fluid sensitizes the frog rectus muscle to acetylcholine (15, 37) and it contracts isolated rat colon preparations (15). The active prin-

ciple has properties of a protein of complex nature. It seems to consist of the active principle proper, an alcohol-soluble compound, bound to the protein moiety (15). When liberated the alcohol-soluble compound moves towards the anode in electrophoresis, indicating an acidic substance.

A similar substance was found by Zetler in brain extracts (93). Its pharmacological action—stimulation of rabbit and guinea pig intestine—can be observed only after treatment of the brain extract with trypsin. This again suggests a complex formed by the active substance and protein-like material.

*6. Plain muscle stimulant in the menstruum.* Acetone or chloroform extracts of menstrual fluid produce a slow, atropine-resistant contraction of rabbit, rat, or guinea pig intestine and rabbit uterus (72). These extracts, in addition to their direct stimulating effect, increase the sensitivity of the test preparations to other stimulating agents such as histamine and acetylcholine. The sensitizing action and lipid-solubility may indicate a lipid-soluble acid.

*7. Unidentified substance in skin.* The skin of rabbit ears contains an acetone-soluble substance which contracts the rat colon preparation in the presence of atropine and lysergic acid diethylamide (9). The active principle is possibly an acid, since in paper electrophoresis at pH 8.8 it moves towards the anode, but in view of the rather alkaline pH this does not prove a predominantly acidic nature.

## III. GENERAL CHARACTERISTICS OF THE LIPID-SOLUBLE ACIDS

It is apparent from the foregoing description that the lipid-soluble acids stimulate smooth muscle. There are only two exceptions, namely, the relaxing action of prostaglandin on the uterine tubes in rabbits (12) and the inhibiting effect of certain samples of Darmstoff on the guinea pig ileum (89). The latter action is probably due to an altered Darmstoff component, *i.e.*, an acidic phospholipid that in its lipid moiety has acquired a lysolecithin-like structure (III, V). Such a structure gives rise to smooth muscle-inhibiting actions, thereby masking the smooth muscle-stimulating action of this Darmstoff component, in organs which are susceptible to the inhibiting action.

A stimulating action upon smooth muscle accounts for the fall in blood pressure caused by intravenous injection of some of these acids. Prostaglandin constricts lung and liver vessels (30) and a similar mechanism is likely to be responsible for the depressor effect of unsaturated fatty acids.

The stimulating action of the lipid-soluble acids on smooth muscle shows characteristic features. The muscle contracts slowly and usually after the acid is washed out the sensitivity of the tissue to other smooth muscle-stimulating agents such as histamine, acetylcholine and bradykinin greatly increases. Further, different smooth muscle preparations are nearly equally sensitive to most of the acids. Thus, unlike the biologically active amines which show a wide range of effects the lipid-soluble acids represent a group of substances with very similar biological effects. This suggests that their actions depend mainly on those features which are common to all acids in the group, namely, lipid-solubility and acidic nature.

Unsaturation seems to be essential for the biological activity of the lipid-soluble acids. Thus, prostaglandin becomes inactive on hydrogenation (14) and the action of irin is lost after bromination (7). Several saturated fatty acids tested have been found to be inactive on the guinea pig ileum (39, 86, 90). Unsaturation causes increased solubility of a lipid compound and this may be one reason why Darmstoff, prostaglandin and irin are highly active. A saturated phosphatidic acid, L-$\alpha$-(dimyristoyl)glycerophosphate, was found to contract the rabbit duodenum, but was about 1000 times less active than purified Darmstoff (90). On the other hand, the unsaturated fatty acids show that unsaturation *per se* is not sufficient but that the position and steric configuration of the double bond is significant. Oleic acid, for instance, is inactive on smooth muscle organs (33, 86) and so is *trans*-vaccenic acid (64). This specificity raises the question whether any reactions at the site of the double bonds are involved in the biological action of unsaturated fatty acids. Oxidative reactions are indicated, because of the ease with which unsaturated fatty acids are oxidized and because of the role of essential fatty acids in biochemical oxidation processes. The positive inotropic effect of unsaturated lactones on heart muscle has been found to be associated with peroxides formed (70). Preliminary experiments conducted to find out whether peroxides of unsaturated fatty acids are the active agents for stimulation of the guinea pig ileum, have so far been inconclusive (86).

In irin another structural element seems to favour smooth muscle activity, namely, a hydroxyl group standing in such a position relative to the carboxyl group that formation of a lactone is possible (XI, XII) (7). This recalls to mind that in digitalis glycosides, which also have smooth muscle-stimulating properties, a lactone ring is essential. There is the possibility that prostaglandin, which is another hydroxy acid may form a lactone.

The haemolytic action of some of the lipid-soluble acids bears no relation to their stimulating activity on smooth muscle, but is a property of unsaturated fatty acids regardless of whether they stimulate smooth muscle. It is not dependent on acidic nature but probably on solubilizing properties which are particularly pronounced in lysolecithin (91).

## IV. DISTINCTION OF THE LIPID-SOLUBLE ACIDS FROM OTHER SIMILARLY ACTING COMPOUNDS AND FROM ONE ANOTHER

A convenient method for separating amines and lipid-soluble acids is partition of the material between water and a suitable solvent at acid pH. The hydrophilic amines and peptides are left in the aqueous phase whereas the lipid-soluble acids are concentrated in the organic solvent layer. However, it must be realized that partition is not a complete extraction of a compound by one of a pair of solvents but a division of its total amount into two proportions, one in each phase of the solvent system. The ratio of the concentrations in the two phases depends on the partition coefficient of the compound. On distribution between water and an organic solvent, only a small proportion of a hydrophilic amine or peptide goes into the organic solvent layer at acid pH, but this amount may, under unfavourable conditions, be sufficient to exert a pronounced effect when tested on an iso-

lated smooth muscle preparation. Therefore it is necessary to compare both phases obtained after partition. If the organic layer is more active than the aqueous layer, the main active principle is a lipid-soluble substance. If the reverse is true, the presence of a lipid-soluble acid can be neither excluded nor postulated with certainty. In such a case countercurrent distribution will give additional information showing several maxima of activity if a mixture of amines and acids is present. For similar reasons the observation, that an unknown biologically active compound is extracted by solvents from dry residues does not *per se* give a clear indication of its nature, as traces of extracted material may suffice to give strong effects if the compound is highly active.

Another complication may arise from the formation of a complex of a lipid-soluble acid with a watersoluble cation. In the author's laboratory a gut-stimulating compound was extracted from intestine which after partition between dilute HCl and butanol was found predominantly in the aqueous layer, indicating a hydrophilic base. However, after repeating the same experiment in the presence of the chelating agent, EDTA, about 90% of the biological activity was found in the butanol layer. Probably the extract contained a lipid-soluble acid which was linked to a cationic compound forming a complex that was predominantly water-soluble. The acid was liberated after binding of the basic component by EDTA and its true behaviour unmasked.

Another problem is that of distinguishing one lipid-soluble acid from another. With amines special test organs are used for biological assay which react almost specifically to one compound, and advantage is taken of specific inhibitors. Chemical purification is then often not required. For the biological actions of the lipid-soluble acids specific tests are usually not available and specific antagonists are unknown. At present, it is therefore necessary to characterize a lipid-soluble acid by physico-chemical properties and to use the bioassay only for the detection of active fractions and for the quantitative estimation of purified material.

Two separate groups of pharmacologically active lipid-soluble acids can be formed, acidic phospholipids (Darmstoff) and unsaturated fatty acids (G-acid, *cis*-vaccenic acid, SRS-C). They can be separated by countercurrent distribution in the system of phosphate buffer-methanol-butanol-benzene described on page 413, or by paper chromatography. For example, in chromatography with benzene or petroleum ether the phospholipids do not move on paper, whereas the unsaturated fatty acids run with the solvent front. In some instances, Darmstoff and unsaturated fatty acids are different in their pharmacological actions. The response of the isolated guinea pig ileum to Darmstoff is the same on repeated administration, but this organ becomes progressively insensitive to unsaturated fatty acids under similar conditions. Further, in contrast to Darmstoff, unsaturated fatty acids have a hypotensive effect in rabbits.

Prostaglandin is presumably a hydroxy-fatty acid, different from both acidic phospholipids and simple unsaturated fatty acids. However, there are similarities between prostaglandin and Darmstoff in addition to features common to all lipid-soluble acids. In paper chromatography with methylethylketone-di-

ethylamine-water mixture prostaglandin has the same $R_f$-value as Darmstoff (0.40 to 0.45) (25, 83). Unsaturated fatty acids move much faster with this solvent. Chemical properties suitable for the separation of prostaglandin and Darmstoff are, at present, unknown. Pharmacologically, prostaglandin also resembles Darmstoff. In a few experiments the two compounds were tested on the rabbit and guinea pig gut. The ratio of activity of the two substances was the same in both preparations (90). Further, on the guinea pig gut the action of small doses of prostaglandin was depressed by atropine as was that of Darmstoff. This is in contrast to the observation of Eliasson (25), who found the response of the guinea pig gut to prostaglandin to be resistant to atropine. The reason for the discrepancy is not known. Differences in the pH of Tyrode solution might alter the effectiveness of atropine as in the antagonism $BaCl_2$-atropine (24). Differences in the dosage used might also account for the discrepancy. The antagonistic action of atropine is more pronounced with low doses of Darmstoff than with high doses (89), and this may be so with prostaglandin.

On the arterial blood pressure the action of prostaglandin differs from that of Darmstoff and resembles more closely that of unsaturated fatty acids. The accessory genital glands of some species contain vesiglandin instead of prostaglandin. As vesiglandin differs from prostaglandin by a lower ratio of smooth muscle-stimulating to depressor activity, and as Darmstoff lacks depressor activity one is tempted to assume two active principles: a depressor one, the main principle in vesiglandin, and a smooth muscle-contracting compound which may be identical with Darmstoff. Prostaglandin could be a mixture of both. This assumption has been rejected by Euler on the basis of his experiments (29). It should be noted that vesiglandin has not been extensively investigated and it may be quite different from prostaglandin. However, the possibility that two effects of an unknown compound are due to a mixture of two substances should always be kept in mind and this applies particularly to lipid-soluble acids because of the difficulty often encountered in separation of lipids.

Irin can be distinguished from Darmstoff by the different $R_f$-values of the two substances in paper chromatograms run with methylethylketone-diethylamine-water [Darmstoff $R_f$ 0.4 (83); irin $R_f$ 0.9 (8)]. That irin does not give a positive phosphate reaction could be misleading, since a negative reaction occurs with Darmstoff when too little material is used for the test. The biological assay is far more sensitive for the detection of Darmstoff than the chemical test for phosphate. The high $R_f$-value of irin in chromatograms developed with methylethylketone-diethylamine is similar to that of fatty acids, but irin can be distinguished by the reaction with diphenylcarbazide which indicates a hydroxyl group (7). As the biological activity is lost after the treatment with the reagent, the reaction is given by the active material itself. The character of an unsaturated hydroxy-fatty acid brings irin into close relationship to prostaglandin, which, curiously enough, behaves differently in paper chromatograms run with methylethylketone-diethylamine, having a far lower $R_f$-value than irin. One would expect more closely related $R_f$-values from similar compounds, isomers or

homologues, in a solvent mixture incapable of separating isomers or homologues of the normal fatty acid series. The difference in mobility on paper may indicate that one of the two substances (prostaglandin?) has a more complicated structure than that of a simple hydroxy-fatty acid. This difference could also be explained by assuming irin to be present as a lactone and prostaglandin as free acid. However, in the solvent mixture used for the chromatography irin is not likely to be present as the lactone but rather as the free acid.

Fraction X and SRS-A are, at present, not characterized sufficiently to delineate their relationship to the lipid-soluble acids already mentioned, and the same applies to the substances headed "substances possibly related to lipid-soluble acids".

## V. PHYSIOLOGICAL SIGNIFICANCE OF LIPID-SOLUBLE ACIDS

Widely diverse functions have been suggested for lipid-soluble acids extracted from tissues. Prostaglandin could by motor effects on the male and female genital tract or accessory glands facilitate impregnation. Darmstoff is assumed to be involved in the physiological movements of the gut. Irin possibly plays a role in the function of the iris. *Cis*-vaccenic acid in erythrocytes or plasma, could regulate ageing of erythrocytes by promoting haemolysis. The slow reacting substances SRS-A and SRS-C possibly are significant in cell injury produced by anaphylaxis or venoms.

With the exception of the systemic actions of SRS-A and SRS-C the functions attributed to the lipid-soluble acids are those of local hormones. In some instances, liberation of a lipid-soluble acid by nervous stimulation seems possible or probable. Whether this means that lipid-soluble acids could act as neurohumoral transmitters seems doubtful, as it is hard to assume that they could replace functions which otherwise are exerted by hydrophilic bases, such as acetylcholine or adrenaline. The liberation of a lipid-soluble acid by nervous stimulation is more likely an indirect event connected with, but not strictly a part of, the process of transmission, or the lipid-soluble acids are involved in the transmission at another step of the process than are the conventional transmitters.

Physiological functions attributed to a given lipid-soluble acid are usually concerned with that tissue in which the compound has been found, but there are indications that some of the lipid-soluble acids do occur in more than one tissue. *Cis*-vaccenic acid, besides being found in blood, occurs abundantly in brain, and a substance possibly related to Darmstoff occurs in skeletal muscle and in brain.

Such findings are difficult to reconcile with the idea of a specific function; they suggest that the lipid-soluble acids may exert a more general function than would be expected from their occurrence in certain tissues only. It is apparent that the lipid-soluble acids are substances with the general property of stimulating smooth muscle. Stimulation of excitable cells is brought about by depolarization, that is, by equilibration of concentration differences of cations inside and outside the cell. It is conceivable that acids capable of forming lipid-soluble salt complexes with cations would increase the rate of passage of cations through the cellular

membranes, when experimentally applied to a tissue. They would thus produce depolarization by overcoming the action of the "sodium pump" which during rest or recovery establishes the ionic potential difference. Phosphatidic acids are known to form lipid-soluble complexes with cations such as sodium and potassium (55). Alkali salts of fatty acids are also lipid-soluble, although to a lesser degree than those of phosphatidic acids.

Another possibility would be that lipid-soluble acids experimentally applied impair the passage of cations through cellular membranes by competition with transport mechanisms. It is interesting to note that various lactones, especially cardiac glycosides, interfere with potassium influx and sodium efflux (53, 54, 78), probably by inhibiting specifically the carrier mechanism (60). A lactone configuration has been postulated for irin (7) and might be considered for prostaglandin.

The endogenous lipid-soluble acids may also affect cation transport and this may be their basic function in various organs, as the same fundamental processes underlie excitability and excitation wherever these occur. However, whereas lipid-soluble acids artificially applied probably increase permeability to cations unspecifically and tend to cancel existing concentration differences, it is tempting to assume that endogenous lipid-soluble acids—at least acidic phospholipids—if fixed into cellular membranes are involved in the specific transport of sodium ions out of the cell, thus keeping or rendering the cell excitable. As a working hypothesis the following cycle is suggested. A phosphatide, *e.g.*, lecithin, is split by an enzyme system situated at the inner side of the membrane, into phosphatidic acid and choline. The phosphatidic acid combines with sodium ion; this salt and the choline with the concentration gradient move across the membrane towards the outside. Another enzyme system situated at the outside of the membrane resynthesizes lecithin from phosphatidic acid and choline, thereby liberating the sodium which is thus carried out of the cell. The lecithin would move back to the inside and complete the whole process in a cycle. Several facts and assumptions in support of this hypothesis and others, at present, difficult to reconcile with the hypothetical transport mechanism, are discussed elsewhere (89).

The problem of sodium extrusion can be viewed in another way. One might ask, how could a carrier in living matter combine with sodium if not by an acidic group? Further, is lipid-solubility not the most favourable property for diffusion (outward) of the sodium-carrier complex enabling it to more than compensate for the passive diffusion (inward) of free sodium ions? Since phosphatides are abundantly present in membranes, phosphatidic acids which can be regarded as derivatives or precursors of phosphatides would lend themselves to this function. The work of Hokin and Hokin (50, 51), showing increased phospholipid turnover on excitation of gland and nerve cells, would support this assumption. However, theories of ion transport have been developed which do not involve any carrier mechanism (79) and the hypothetical nature of the suggested transport mechanism should be borne in mind.

## REFERENCES


1. AMBACHE, N.: The action of "Darmstoff" on the rabbit ileum. J. Physiol. 125: 38P, 1954
2. AMBACHE, N.: The use and limitations of atropine for pharmacological studies on autonomic effectors. Pharmacol. Rev. 7: 467–494, 1955.
3. AMBACHE, N.: Irin, a smooth-muscle contracting substance present in rabbit iris. J. Physiol. 129: 65–66P, 1955.
4. AMBACHE, N.: Trigeminomimetic action of iris extracts in rabbits. J. Physiol. 132: 49–50P, 1956.
5. AMBACHE, N.: Properties of irin, a physiological constituent of the rabbit iris. J. Physiol. 135: 114–132, 1957.
6. AMBACHE, N.: A rapid purification of irin and the use of the hamster colon for its assay. J. Physiol. 138: 6–7P, 1957.
7. AMBACHE, N.: The unsaturated nature of irin and its interaction with lactones. J. Physiol. 140: 24–25P, 1958.
8. AMBACHE, N.: Personal communication.
9. AMBACHE, N. AND WEST, G. B.: Presence of an unidentified pharmacologically active substance in skin extracts. J. Physiol. 133: 19–20P, 1956.
10. ARMSTRONG, D., JEPSON, J. B., KEELE, C. A. AND STEWART, J. W.: Pain producing substance in human inflammatory exudates and plasma. J. Physiol. 135: 350–370, 1957.
11. ASPLUND, J.: A quantitative determination of the content of contractive substances in human sperm and their significance for the motility and vitality of the spermatozoa. Acta physiol. scand. 13: 103–108, 1947.
12. ASPLUND, J.: Some preliminary experiments in connection with the effect of prostaglandin on the uterus and tubae *in vivo*. Acta physiol. scand. 13: 109–114, 1947.
13. BERALDO, W. T.: Formation of bradykinin in anaphylactic and peptone shock. Amer. J. Physiol. 163: 283–289, 1950.
14. BERGSTRÖM, S.: Prostaglandinets kemi. Nord. med. 42: 1465–1466, 1949.
15. BHATTACHARYA, B. K., FELDBERG, W. AND VOGT, W.: Some properties of the substance in human cerebrospinal fluid sensitising the frog rectus muscle to acetylcholine. J. Physiol. 137: 460–472, 1957.
16. BOVET, D.: Personal communication.
17. BOVET, D., BOVET-NITTI, F., CANTORE, G. P., CASINOVI, G. C., LONGO, V. G., MARINI-BETTOLO, G. B., RENZI, L. AND ROGERS, E. F.: Sur un nouveau principe contracturant isolé de la méduse *Rhizostoma pulmo* L. Rev. suisse Zool. 62: 94–101, 1955.
18. BOYER, P. D.: Fat-soluble vitamins. Essential fatty acids. Annu. Rev. Biochem. 24: 481–496, 1955.
19. BROCKLEHURST, W. E.: Occurrence of an unidentified substance during anaphylactic shock in cavy lung. J. Physiol. 120: 16P, 1953.
20. BROCKLEHURST, W. E.: Response of the cavy ileum to "SRS-A" from lung of man and of cavy. J. Physiol. 128: 1P, 1955.
21. BROCKLEHURST, W. E.: A slow reacting substance in anaphylaxis—"SRS-A". In: Ciba Foundation Symposium on histamine, pp. 175–179. Little, Brown and Co., Boston 1956.
22. BROCKLEHURST, W. E.: Personal communication.
23. CARLSTEN, A., KAHLSON, G. AND WICKSELL, F.: Accumulation in the canine lymph during reactive hyperaemia of a substance contracting the guinea pig's gut which is not histamine. Acta physiol. scand. 17: 384–394, 1949.
24. DAHLBOM, R., EDLUND, T., EKSTRAND, T. AND LOHI, A.: The activity of 10-aminoacylphenothiazines, basically substituted derivatives of phenothiazine-10-carboxylic acid, atropine, papaverine and some other spasmolytic drugs against barium- and acetylcholine-induced spasms of guinea-pig and rat ileum. Acta pharm. tox., Kbh. 9: 168–178, 1953.
25. ELIASSON, R.: A comparative study of prostaglandin from human seminal fluid and from prostate gland of sheep. Acta physiol. scand. 39: 141–146, 1957.
26. ELLIS, S.: The pharmacologic analysis of intestinal stimulants. Science 114: 325–326, 1951.
27. EULER, U. S. VON: Zur Kenntnis der pharmakologischen Wirkungen von Nativsekreten und Extrakten männlicher accessorischer Geschlechtsdrüsen. Arch. exp. Path. Pharmak. 175: 78–84, 1934.
28. EULER, U. S. VON: Über die spesifische blutdrucksenkende Substans des menschlichen Prostata- und Samenblasensekretes. Klin. Wschr. 14: 1182–1183, 1935.
29. EULER, U. S. VON: On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). J. Physiol. 88: 213–234, 1937.
30. EULER, U. S. VON: Weitere Untersuchungen über Prostaglandin, die physiologisch aktive Substanz gewisser Genitaldrüsen. Skand. Arch. Physiol. 81: 65–80, 1939.
31. EULER, U. S. VON: Prostaglandinets autofarmakologiska verkningar. Nord. med. 42: 1463–1465, 1949.
32. EULER, U. S. VON AND HAMMARSTRÖM, S.: Über das Vorkommen des Prostaglandins in Tierorganen. Skand. Arch. Physiol. 77: 96–99, 1937.
33. FELDBERG, W., HOLDEN, H. F. AND KELLAWAY, C. H.: The formation of lysocithin and of a smooth muscle-stimulating substance by snake venoms. J. Physiol. 94: 232–248, 1938.
34. FELDBERG, W. AND KELLAWAY, C. H.: Circulatory effects of the venom of the Indian cobra (*Naia naia*) in cats. Aust. J. exp. Biol. med. Sci. 15: 159–172, 1937.
35. FELDBERG, W. AND KELLAWAY, C. H.: Circulatory effects of the venom of the Indian cobra (*Naia naia*) in dogs. Aust. J. exp. Biol. med. Sci. 15: 441–460, 1937.
36. FELDBERG, W. AND KELLAWAY, C. H.: Liberation of histamine and formation of lysocithin-like substances by cobra venom. J. Physiol. 94: 187–226, 1938.
37. FELDBERG, W., MALCOLM, J. L. AND SHERWOOD, S. L.: Some effects of tubocurarine on the electrical activity of the cat's brain. J. Physiol. 132: 130–145, 1956.

37a. Feulgen, R. and Bersin, Th.: Zur Kenntnis des Plasmalogens. IV. Mitteilung. Eine neuartige Gruppe von Phosphatiden (Acetalphosphatide). Hoppe-Seyl. Z. **260**: 217–245, 1939.
38. Freund, H.: Über die pharmakologischen Wirkungen des defibrinierten Blutes. Arch. exp. Path. Pharmak. **86**: 266–280, 1920.
39. Gabe, Y.: Observations on a substance in human plasma which gives a slow contraction of guinea pig gut *in vitro*. Brit. J. Pharmacol. **11**: 93–98, 1956.
40. Gayer, J. and Sarre, H.: Untersuchungen über die vasoconstrictorische Aktivität im Plasma essentieller Hypertoniker. Klin. Wschr. **34**: 334–335, 1956.
41. Goldblatt, M. W.: Properties of human seminal plasma. J. Physiol. **84**: 208–218, 1935.
42. Habermann, E.: Zur pharmakologischen Charakterisierung elektrophoretischer Fraktionen der Gifte von *Naja nigricollis* und *Naja haje*. Hoppe-Seyl. Z. **197**: 104–107, 1954.
43. Habermann, E.: Beiträge zur Pharmakologie von Phospholipase A. Arch. exp. Path. Pharmak. **230**: 538–546, 1957.
44. Habermann, E. and Neumann, W.: Die Hemmung der Hitzekoagulation von Eigelb durch Bienengift—ein Phospholipase-Effekt. Hoppe-Seyl. Z. **297**: 179–193, 1954.
45. Hanes, C. S. and Isherwood, F. A.: Separation of the phosphoric esters on the filter paper chromatogram. Nature, Lond. **164**: 1107–1112, 1949.
46. Hanon, F., Coquoin-Carnot, M. and Pignard, P.: Nouvelles recherches sur l'eutocine, substance ocytocique, lipidique, extrait de l'utérus et du liquide amniotique au cours de l'accouchement. Ann. Endocr., Paris **14**: 204–219, 1953.
47. Hardwick, D. C.: Personal communication.
48. Hilditch, T. P.: The chemical constitution of natural fats. London 1949.
49. Hilton, S. M. and Lewis, G. P.: The mechanism of the functional hyperaemia in the submandibular salivary gland. J. Physiol. **129**: 253–271, 1955.
50. Hokin, L. E. and Hokin, M. R.: Effects of acetylcholine on phosphate turnover in phospholipides of brain cortex *in vitro*. Biochim. biophys. acta **16**: 229–237, 1955.
51. Hokin, L. E. and Hokin, M. R.: Effects of acetylcholine on the turnover of phosphoryl units in individual phospholipids of pancreas slices and brain cortex slices. Biochim. biophys. acta **18**: 102–110, 1955.
52. Huennekens, F. M., Hanahan, D. J. and Uziel, M.: Paperchromatography of lecithins. J. biol. Chem. **206**: 443–447, 1954.
53. Kahn, J. B.: Effects of various lactones and related compounds on cation transfer in incubated cold-stored human erythrocytes. J. Pharmacol. **121**: 234–251, 1957.
54. Kahn, J. B. and Acheson, G. H.: Effects of cardiac glycosides and other lactones, and of certain other compounds, on cation transfer in human erythrocytes. J. Pharmacol. **115**: 305–318, 1955.
55. Kates, M.: Hydrolysis of lecithin by plant plastid enzymes. Canad. J. Biochem. Physiol. **33**: 575–589, 1955.
56. Kekwick, R. A. and Mackay, M. E.: Quoted from (39).
57. Kellaway, C. H. and Trethewie, E. R.: The liberation of a slow-reacting smooth muscle-stimulating substance in anaphylaxis. Quart. J. exp. Physiol. **30**: 121–145, 1940.
58. Khairallah, P. A.: Personal communication.
59. Kuck, H. and Vogt, W.: Pharmakologische Wirkungen des Darmstoffs. Arch. exp. Path. Pharmak. **209**: 71–81, 1950.
60. Kunz, H. A. and Sulser, F.: Über die Hemmung des aktiven Kationentransportes durch Herzglykoside. Experientia **13**: 365–367, 1957.
61. Laser, H.: A method of testing the antimalarial properties of compounds *in vitro*. Nature, Lond. **157**: 301, 1946.
62. Laser, H.: Haemolytic system in the blood of malaria-infected monkeys. Nature, Lond. **161**: 560, 1948.
63. Laser, H.: The isolation of a haemolytic substance from animal tissues and its biological properties. J. Physiol. **110**: 338–355, 1950.
64. Laser, H.: Personal communication.
65. Laser, H. and Friedmann, E.: Crystalline haemolytic substance from normal blood. Nature, Lond. **156**: 507, 1945.
66. Le Heux, J. W.: Cholin als Hormon der Darmbewegung. Pflüg. Arch. ges. Physiol. **173**: 8–27, 1919.
67. Lewis, G. P.: Formation of plasma kinins by plasmin. J. Physiol. **40**: 285–300, 1958.
68. Lewis, G. P. and Work, T.: Formation of bradykinin or bradykinin-like substances by the action of plasmin on plasma proteins. J. Physiol. **135**: 7–8P, 1957.
69. Masson, G. M. C., Helmcke, G., Corcoran, A. C. and Page, I. H.: Pressor and myotrophic activities of incubated plasma. Proc. Soc. exp. Biol., N.Y. **94**: 544–546, 1957.
70. Mendez, R.: Pharmacology and chemistry of substances with cardiac activity. III. The effect of simple unsaturated lactones and *t*-butyl hydrogen peroxide on the isolated frog heart. J. Pharmacol. **81**: 151–159, 1944.
71. Morton, I. D. and Todd, A. R.: The haemolytic acid present in horse brain. 1. Purification and identification as cis-octadec-11-enoic acid. Biochem. J. **47**: 327–330, 1950.
72. Pickles, V. R.: A plain muscle stimulant in the menstruum. Nature, Lond. **180**: 1198–1199, 1957.
73. Rapport, M. M.: Serum vasoconstrictor (serotonin). V. The presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. J. biol. Chem. **180**: 961–969, 1949.
73a. Rapport, M. M., Lerner, B., Alonzo, N. and Franzl, R. E.: The structure of plasmalogens. II. Crystalline lysophosphatidal ethanolamine (acetal phospholipide). J. biol. Chem. **225**: 859–867, 1957.
74. Rocha e Silva, M. and Beraldo, W. T.: Dynamics of recovery and measure of drug antagonism. Inhibition of smooth muscle by lysocithin and antihistaminics. J. Pharmacol. **93**: 457–469, 1948.
75. Rocha e Silva, M., Beraldo, W. T. and Rosenfeld, G.: Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Amer. J. Physiol. **156**: 261–273, 1949.

76. Rouser, G., Marinetti, G. V., Witter, R. F., Berry, J. F. and Stotz, E.: Paper chromatography of phospholipides. J. biol. Chem. 223: 485–497, 1956.
77. Schachter, M.: A delayed slow contracting effect of serum and plasma due to the release of a substance resembling kallidin and bradykinin. Brit. J. Pharmacol. 11: 111–118, 1956.
78. Schatzmann, H. J.: Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrozytenmembran. Helv. physiol. acta 11: 346–354, 1953.
79. Schlögl, R.: Materie-Transport durch geladene Porenmembranen. Habilitationsschrift, Göttingen 1957.
80. Vogt, W.: Über die stoffliche Grundlage der Darmbewegungen und des Vagusreizes am Darm. Arch. exp. Path. Pharmak. 206: 1–11, 1949.
81. Vogt, W.: Zum Einfluss biogener Stoffe auf die Darmmotilität. Arch. exp. Path. Pharmak. 219: 138–147, 1953.
82. Vogt, W.: Über die Beziehung des Darmstoffs zur Substanz P. 3. Mitteilung. Arch. exp. Path. Pharmak. 220: 365–377, 1953.
83. Vogt, W.: Eigenschaften und Gewinnung von Darmstoffpräparaten. Arch. exp. Path. Pharmak. 227: 224–233, 1955.
84. Vogt, W.: Zur Wirkungsweise von Darmstoff. Arch. int. Pharmacodyn. 106: 294–306, 1956.
85. Vogt, W.: Untersuchungen zur Natur von H-Substanzen. Arch. exp. Path. Pharmak. 228: 200–201, 1956.
86. Vogt, W.: Pharmacologically active substances formed in egg yolk by cobra venom. J. Physiol. 136: 131–147, 1956.
87. Vogt, W.: Pharmacologically active lipidsoluble acids of natural occurrence. Nature, Lond. 179: 300–304, 1957.
88. Vogt, W.: The chemical nature of Darmstoff. J. Physiol. 137: 154–167, 1957.
89. Vogt, W.: Chemische und pharmakologische Eigenschaften saurer Phospholipide (Darmstoff). Arzneim.-Forsch. 8: 253–263, 1958.
90. Vogt, W.: Unpublished data.
von Euler, U. S. see 27–32.
91. Webster, G. R.: Clearing action of lysolecithin on brain homogenates. Nature, Lond. 180: 660–661, 1957.
92. Weiland, W.: Zur Kenntnis der Entstehung der Darmbewegung. Pflüg. Arch. ges. Physiol. 147: 171–196, 1912.
93. Zetler, G. and Ohnesorge, G.: Die Substanz P-Konzentration im Gehirn bei verschiedenen Funktionszuständen des Zentralnervensystems. Arch. exp. Path. Pharmak. 231: 199–210, 1957.