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CHEMICAL EXCITANTS OF CUTANEOUS PAIN.
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by

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ABSTRACT
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The thesis deals with the production of pain in man by substances of biological origin. Pain has been recorded subjectively. Permanenent graphic records have been obtained. A new technique of administration of test-substances, namely, application to the exposed base of a cutaneous blister induced by cantharidin has been used and its superiority over 'pricking-through' and intra-dermal injection techniques has been demonstrated.

The method allows the study of:-

i. chemical excitation of pain,

ii. chemical antagonism of chemically induced pain,

and iii. local chemical antagonism of mechanically induced pain.

The pain-producing properties of hypo- and hypertonic solutions, acid solutions, acetylcholine, adrenaline, nor-adrenaline, histamine, 5-hydroxytryptamine, adenosine triphosphate, potassium ion, substance P, bradykinin, angiotonin, and related substances have been investigated. Pain production by posterior root extract (Hellauer and Umrah, 1947) platelet-extract, serum, plasma, inflammatory exudates, heated human plasma and of aqueous extract of macerated human skin have been
observed.

Relationship of chemical structure to pain-producing action in acetylcholine-like and 5-hydroxytryptamine-like substances has been studied.

The pain-producing agents in blood, inflammatory exudates and skin-extracts stimulate smooth muscle. The isolated rat-uterus is a particularly useful test-preparation.

Chemical and biological studies have indicated that the active agent of serum is 5-hydroxytryptamine, that that of plasma and inflammatory exudates is a polypeptide and that that of skin extracts is probably of organic acid nature.

The findings account for the pain induced by 'fresh' water (hypotonicity) and sea-water (hypertonicity) on wounds, by wasp venom, nettle-stings and serum. The pain of mechanical and thermal injury to the skin may well be caused through liberation of 5-hydroxytryptamine and of polypeptide and organic acid type substances from broken platelets, plasma, and disrupted skin cells.
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INTRODUCTION
THE NATURE OF PAIN.

Almost without exception, man is familiar with the experience we call 'pain'. It is of interest and concern for two reasons:—(1) because of its supremely unpleasant character; (2) because it warns of damage or danger to the person, induces protective reflexes and courses of action, and so helps in the effort to survive.

The nature of pain and its stimulatory mechanisms has for long been a subject of wonder and controversy. Lacking an obvious end-organ as have the senses of sight, hearing, smell and taste, and not being restricted to any definite part of the body, but rather pervading the whole and being accompanied by the quality of unpleasantness (which itself can also be aroused through various senses, by so-called 'overstimulation' of them), pain seemed to transcend the purely sensory field and to enter the fields of mental life. For Plato and Aristotle it was a passion of the soul. This concept remained with us for long.

The suggestion that pain is a separate and distinct sense is attributed to Avicenna (11th Century) (See Dallenbach, 1939). The nineteenth century saw a revival of curiosity in these matters with a re-affirmation of the Avicenna theory by Lotze (1852) and
a definite formulation of it on an experimental basis by Schiff who incised the spinal cord of animals in such a way as to leave either pain or touch intact, below the level of the incision, but not both. The separation of the senses was in the same way each time, dependent on whether it was the grey or the white matter which he cut, (Schiff, 1858). Aroused clinical interest discovered pathological conditions of the spinal cord (e.g. syringomyelia) with accompanying similar sensory effect (see also Funke, 1879).

It was difficult to drag the human mind away from its age-long concept of pain as being 'just' the mental result of 'overstimulation' of more familiar sensory systems (viz. those concerned with heat and cold, touch and pressure, light, sound, odour). Goldscheider was one of the last adherents of the old idea, believing that excessive stimulation of one particular sense, that of pressure, was responsible for pain. (Goldscheider, 1926). This seemed not unreasonable since excessive pressure or tension in the regions of the various sense-organs will always arouse pain.

The chief opponent of the Goldscheider theory was von Frey who believed that not only was pain a system of sensitivity apart from pressure, warmth and cold but that it was mediated by receptor organs of its own - free nerve endings (von Frey, 1922).
Considerations which occasioned his belief centered round:-

1. distribution of pain points;
2. the differential action of local anaesthetics on pain and other tactual sensitivities;
3. pain threshold;
4. apparent pathological separation of pain from other tactual systems in diseases of the cord and brain;
5. the relatively long latency of pain sensations following stimulation;
6. the apparent yielding of pain sensations only by a tissue (cornea).

There was great controversy between the beliefs of these two men. According to Goldscheider, what is pressure on weak stimulation should yield pain on strong stimulation. One would then expect that pain on being sufficiently weakened should change into pressure. In fact this does not occur unless the pain stimulus is a pressure stimulus as well.

Dallenbach, experimenting with pain induced by pressure, heat or cold, found that each of these agents produced:-

1. the expected sensations dependent on the nature of the individual stimulus;
2. pain, on more intensive stimulation;
3. adaptation to pain, reduction thereof, and the leaving of the respective initial sensation (pressure, heat or cold, dependent on the stimulus
employed.) During the course of the adaptation there was no sensation of pressure unless pressure itself was the pain stimulus. (Dallenbach, 1939).

These observations on adaptation of the pain sense contrafeute the Goldscheider theory and are in fact to be expected if pain is independent of other cutaneous modalities. Thus pain, of prime importance to the well-being and survival of man, is a special sense mediated by special nerve systems.

But what of the sensory end-organ?

von Frey was impressed by the fact that unencapsulated terminals of sensory nerves are very widely distributed throughout the body and that no encapsulated endings have such a wide distribution. This applied to the epidermis, dermis, mucous membrane, somatic and visceral organs and appeared to cover the field of pain sensitivity. (That does not however imply that pain is the only sensation initiated by stimulation of free nerve endings - see Nafe and Wagoner, 1937; Sjöqvist, 1938).

The fact that a sharp stimulus to an extremity can elicit two pains one sharp and pricking and the other burning, well separated in time, suggests that the pain stimulus may be carried by two sets of nerve fibres of differing conducting rates. Weddell (1947) showed that some presumed pain-nerve endings are connected to myelinated nerve (rapidly conducting) and some to unmyelinated (slowly conducting), the
myelinated lying just beneath the epithelium and the non-myelinated lying more deeply in the skin and near tiny blood vessels. He had already shown that agitation of a needle in the skin could arouse 'first' pain only, providing the agitations were in the upper epidermis (0.25 to 0.5 mm). Slow pain occurred when the needle disturbed the lower epidermis and dermis (0.50 to 1.0 mm). (Woollard, Weddell & Harpman, 1940).

Thus the presence of pain in the skin was related to the presence of free nerve endings, and the type of pain elicited (pricking or burning) to the type of nerve fibre to which the endings were connected. The complete pain picture will be expected to be modified by the relative distribution of stimulation between these systems. Derangements of innervation of the skin might well be expected to produce deranged sensation. Weddell and his colleagues found 59 patients with scarred or denervated skin. In all cases where pin-prick elicited particularly unpleasant pain sensations ('protopathic' - Head, 1920), histological examination showed that instead of the fine nerve nets being in overlap and interdigitation they were isolated from their neighbours and a single nerve fibre supplied all the endings in the test region. Where no isolation of terminals, and hence simplification of innervation, were found there was no 'protopathic' pain on pin-prick. Thus the general distribution of stimulation in space affects the pain-
picture as a whole.

**ITCHING.**

Itching can occur in normal and pathological skin. It may be induced chemically, say following certain insect bites, or electrically by repeated shocks which individually elicit no other sensation. (N.B. The threshold for electrical stimulation of pain is in general lower than that for stimulation of temperature or pressure). Weak but supraliminal shocks elicit pain passing to more intense pain on increasing the intensity of the repeated shock stimulations (Bishop, 1943a). Thus itching would seem to be associated with the pain system. That itch and pain follow the same nerve pathways is suggested by the facts that:

1. it is set up by low repetitive electrical stimulation of pain 'spots' on the skin. Itch, non-painful prick and pain are "elicited by appropriate patterns of stimulation from the same point." This qualitative shift points "to a central qualitative interpretation of sensory impulses depending only on quantitative factors involving identical peripheral mechanisms within the single modality of pricking pain" (Bishop, 1943b).

2. (Bickford, 1938).

a. section of the lateral spinothalamic tract in man results in abolition of itch of mechanical or chemical induction, and of pain, though not of touch;
b. the same changes can be observed in cases of spinal tumour and syringomyelia;

c. "spontaneous itching is not felt where pain to pin-prick is dulled. It was conspicuously increased in one case of nerve damage where the response to pin-prick was exaggerated."

3. Those constitutionally insensitive to pain are insensitive to itch also, though not to touch (Kunkle & Chapman, 1943).

In the experiments to be described, in the thesis, itching, following the administration of chemical pain-excitants occurred from time to time and provision was made for its recording in the same way, though on an individual record, as for pain itself.

PAIN INDUCED BY PHYSICAL STIMULI.

Methods.

The production of pain and withdrawal reflexes by physical stimuli have been much used in experimental studies on pain and its relief by drugs (mechanical stimuli; Blix, 1885; Alrutz, 1897; Head, 1920; Frey, 1922; Smith, 1938; Grunthal, 1941; thermal stimuli; Alrutz, 1897; Frey, 1922; Smith, 1938; Hardy, Wolff & Goodell, 1940; Andrews & Workman, 1941; D'Amour & Smith, 1941; electrical stimuli; Leyden, 1864; Techiriew & Wattevilla, 1879; Alrutz, 1897; Martin, Withington & Putnam, 1914; Frey, 1922; Knowlton & Gross, 1943; Lanier, 1943).

Inferences.

It may be that the action of the physical stimuli
on the tissues is to induce the production of pain-provoking chemicals. The suggestion that the immediate pain of injury might arise through the mediation of intrinsic materials within the body was first made by Thunberg and by von Frey (Thunberg, 1902; von Frey, 1922).

Recently Hardy et al., have observed that, in the case of pain induced by heat on the skin, the pain threshold and tissue injury threshold temperatures coincide (about 45°C). This suggests that the pain-stimulating factor might well be damaged tissue proteins (Hardy, Wolff & Goodell, 1952a).

The fact that above threshold, pain is proportional to temperature rather than to the duration of exposure (and tissue injury) suggested to these workers that destruction and 'repair' of protein (or formation and destruction of the pain-producing agent) may be taking place simultaneously. On brief exposure to high temperatures where marked pain is felt, though injury is negligible, production of the pain-producing agent far outweighs, for the moment, its destruction. Thus pain stimulation is related to the rate of protein alteration. Their observations are in keeping with the studies of Henriques on thermal changes of proteins and enzymes in relation to tissue injury (Henriques, 1947) They are also in keeping with the known potentiality of plasma proteins to form and destroy polypeptide pain-
producing material rapidly under suitable conditions (see thesis).

THE PRODUCTION OF PAIN BY CHEMICAL AGENTS.

Further observations. Work up to 1950.

The agency of chemical factors in the production of pain is more clearly seen in the effects of application of strong irritants to the skin, in the actions of hypo- and hypertonic solutions to open wounds, and in the effects of wasp, (Vespa Vulgaris) and nettle, (Urtica Urens) stings.

In addition there are certain pathological types of pain which appear to be provoked by endogenous chemical factors. Acid gastric juice induces pain in patients with peptic ulcer; contractions of ischaemic muscle lead to pain of the intermittent claudication type, which is attributed to accumulation of metabolites (Lewis, Pickering and Rothschild, 1931). The pain of inflammation and the accompanying hyperalgesia have also been ascribed to chemical factors.

That pain and hyperalgesia could come about through the agency of chemicals within the body was suggested by Lewis and Hess in 1933, Rosenthal and Minard, 1939, and by Rosenthal in 1950 and Addis, Jepson and Kellgren in 1950. The hyperalgesic or "susceptible" state is not the immediate result of injury but of ensuing inflammation. It is caused by the liberation of some tissue substance, which acts on the pain nerve
endings" (Lewis & Hess, 1933), and, in certain inflammatory, "painful states the damaged tissues are probably producing substances which stimulate the pain-nerve endings directly and also render them abnormally sensitive to other stimuli such as pressure, tissue tension, and changes in temperature. For disposal of these substances an ample blood-flow is essential. Thus a delicate balance is maintained between the blood-flow through the affected part and the accumulation of these substances in the tissues," (Addis et al., 1950).

Evidence in support of these statements was as follows:-

1. Hyperalgesia is not proportional to the extent of whealing. "It is evidently associated with the process of inflammation, being delayed till clear signs of this are established, a fact very well exemplified in the case of the delayed ultraviolet reaction. It is probably a particular and rather advanced phase of inflammation that is concerned, for the susceptible state is not developed in the early stage of redness and whealing, except in the single instance of injury by heat" (Lewis & Hess, 1933)

2. There is delayed pain following skin injury. The injury may be of various types, e.g. rubbing, scratching, freezing, burning, ultraviolet light lesions. Consider the case of rubbed skin. The initial pain lasts only a second or two. "There is
a significant interval of usually 10 or 15 seconds, sometimes longer, after the strokes have ended, before pain begins, the pain grows in intensity for 15 or 45 sec., declines and is gone after lasting 1 to 3 min." (Lewis & Hess, 1933). It is suggested "that the rubbing causes the discharge of some substance into the intercellular spaces, which, first accumulating and then slowly dispersing, accounts for the curious time relations." Occlusion of circulation of the limb before and during injury results in increased intensity and duration of the delayed pain of injury to the limb. This pain is removed at once by restoration of circulation, then it usually returns somewhat as the hyperaemia of the occlusion subsides. Taken together these facts led Lewis and Hess to conclude that the pain-producing substance is relatively stable and diffusible.

Furthermore the same observers, Lewis and Hess, induced pain in injured tissue (areas of friction or ultraviolet light burns) by similar circulatory occlusion, again indicating the presence of chemical transmitters of pain at the site of injury.

3. Similarly, "skin is not brought to the hyperalgesic state at once by mechanical injuries, but only gradually and after an interval of minutes or even hours has elapsed. Thus it cannot be due to
damage of nerve endings occurring at the time of injury and lasting, but must result from something which arises out of injury after a lesser or greater period of delay" (Lewis & Hess, 1933). (Underlining mine).

4. The fact that application of mild warmth (say 32-34°C) to injured skin in the hyperalgesic state causes burning pain irrespective of the manner of initial injury suggests that there is a **common factor** in these states.

5. When an injured limb is in a hyperalgesic state, due to inflammatory tissue reaction (rather than to peripheral nerve injury), pain usually becomes severe following occlusion of the circulation of the limb. Such pain is sometimes the direct result of mechanical interference such as increased turgidity or increased arterial pressure on susceptible nerves (Lewis & Hess, 1933). That it can also increase due to accumulation of a **pain-producing substance** is suggested by the observations of Addis et al. (1950) on heat and occlusion on infected finger-tips. The occluded hyperalgesic finger-tip was placed in water at 40°C till pain was just observed, and then immediately transferred to water at 30°C when pain ceased. The pain slowly returned at this low temperature. Further lowering of temperature (20°C) again produced temporary relief of pain but lowering of temperature still
further than gave no relief and the pain continued
to increase till the circulation was once more
restored by release of the occlusion cuff. If
during such an experiment the pain-free finger-tip
were transferred back to a higher temperature pain
returned immediately.

Thus it appeared that a steady accumulation of
'metabolite' was taking place increasing the
sensitivity of the finger-tip to warmth-induced
pain, pain being felt at lower temperatures as the
occlusion period and concentration of 'metabolite'
increased. Thus, of hyperalgesias of infection,
Kellgren et al. say "The infected finger behaves
like the damaged skin of Lewis and Hess".

"Furthermore, in deep suppuration a collection of
pus may act as a reservoir for pain-producing
substances and the relief of pain which frequently
follows incision and drainage may be due more to
the emptying of this reservoir than to the relief
of tension, although this undoubtedly plays some
part" (Addis et al., 1950).

This concept of production of hyperalgesia and
pain resulting from accumulation of metabolites in
injured tissue could well be linked with modern
views concerning local hormones (Feldberg & Schilf,
1930; Feldberg, 1955). The local hyperalgesic
effect in the area of tissue damage is referred to
as 'primary hyperalgesia'. (Note that this is
to be distinguished from the secondary hyperalgesia which occurs in intact skin proximally, away from the injured tissue, once thought to be directly or indirectly chemically mediated (Lewis, 1937), and which is now attributed to events within the spinal cord (see Hardy, Wolff & Goodell, 1952b).

Workers in this field of chemical stimulation of pain have shown that pain nerves can be stimulated by a variety of applied chemicals and that the hyperalgesic state also, can be induced by their introduction into the body. This previous work has involved:

1. the application of chemicals, such as ether, chloroform, menthol, to the intact skin in man (Rollett, 1899; Alrutz, 1909; Lebermann, 1922; Frey, 1922);

2. the injection of materials (such as acetylcholine, acetylbeta-methylcholine, atropine, histamine, KCl, hypertonic solutions, solutions of various pH's, human skin extract) intradermally, (Emmelin & Feldberg, 1947b; Skouby, 1951; Rosenthal and Minard, 1939; Lewis, 1936a).

3. the injection of CaCl₂ to the periosteum of the tibia (Kellgren & McGowan, 1948).

4. a. the administration of acids, and the subjects' own partially-digested meals, to the stomach in cases of peptic ulcer and the production pain thereby. Subcutaneous injection of histamine had the same pain-producing effect:
b. the relieving of the pain by the removal of gastric contents or by their neutralisation.

c. failure to obtain these effects in normal persons or gastric ulcer cases in remission. (See – Palmer, 1926, 1927; Palmer and Heinz, 1934; Bonney & Pickering, 1946).

5. the direct application of chemicals to:

a. a duodenal ulcer, in a patient, under local anaesthesia, with ulcer exposed and the production of (1), pain by acid administration, and (2), antagonism of mechanically induced and of HCl induced pain by administration of alkali (Dragstedt & Palmer, 1932).

b. 'imitation gastric ulcers' on the forearm (made by shaving off the epidermis and using before and after scabbing and before and after painting with mucus) and the observation of the effect (intensity and time of onset of pain) following, NaCl, HCl and NaOH solution administration (Bonney & Pickering, 1946).

c. finger incisions, by means of a brush (Grützner, 1894). Various halides (NaCl, NaBr, NaI), acids, halogenated acids, alkali's were applied to the wounds in high concentration. Pain was obtained and reaction-time (i.e. duration of delay between application and effect) was used as the index of effect.

d. skin ablations of the forearm – Grützner (1894)
(also Bonney & Pickering, 1946, above).

e. the base of a blister accidentally induced on himself by hot metal - Grützner, 1894, who satisfied himself that the observations obtained in this way agreed with those obtained from various incision wounds and did not pursue the studies on the blister base.


The results of this work have shown that pain can in many instances be chemically induced and suggest that chemicals arising within the body are apparently responsible for pain and hyperalgesia in several clinical or experimental conditions of 'intact' man (viz. i. peptic and duodenal ulcers,

ii. hyperalgesia following

a. traumatic injury,

b. freezing,

c. burning,

d. infection,

iii. deep hyperalgesia with cold-pain,

iv. ischaemic muscle pain especially after exercise),

and among the previous experiments on the actions of the various agents in producing pain those concerning the effects of the following are amongst the most
1. **Tonicity.** Hypertonicity. x 3 isotonic NaCl, administered by intradermal injection was threshold for pain-production (Lewis, 1936a).

2. **pH.** Solutions of pH's 5.8 to 8, given by intradermal injection, do not cause pain per se (Lewis, 1936a). Solutions of pH 1 (e.g. N/10 HCl) applied to ablated epidermis (Bonney & Pickering, 1946) or to peptic or duodenal ulcer (Palmer, 1926, 1927; Bonney & Pickering, 1946; Dragstedt & Palmer, 1932) cause pain.

3. **ACh.** Acetylcholine in concentration of $10^{-2} \text{g/ml.}$ will cause pain when pricked through the intact skin in the presence of histamine (Emmelin & Feldberg, 1947b).

4. **Histamine** is said to cause pain by intradermal injection of concentrations down to $10^{-18} \text{g/ml.}$ (Rosenthal & Sonnenschein, 1948). According to Lewis (1936a) and Emmelin & Feldberg (1947b) $10^{-3} \text{g/ml.}$ histamine pricked through the skin does not cause pain. (It may induce pain when present along with acetylcholine — see (3) above). (Broadbent, 1953, has found that the actions of histamine, whether it will produce itch or pain, depend largely on whether it acts in the epidermis or the dermis).

5. **KCl.** Administered by intradermal injection, 50% of the isotonic concentration, $1.15 \times 10^{-2} \text{g/ml.}$ (in isotonic solution) causes pain, though 25% is inactive (Lewis, 1936a). Applied to the exposed blister base, Grützner, 1894, induced pain by 33% isotonic KCl.
solution and increasingly painful effects by higher concentrations.

6. Human skin extract causes pain on intradermal injection. The pain is delayed and is not due to tonicity, pH, protein, potassium, histamine or acetylcholine (Lewis, 1936a).

Present Work

It seemed worthwhile to expand the knowledge of chemical excitation of pain, and to try to discover and evaluate which of those materials which arise within the body, in health and disease, can cause pain in suitable circumstances. To do so required a suitable experimental method.

The first decision to be made was the choice of experimental species. Since pain is a subjective phenomenon of which "we have no knowledge"...."beyond that derived from human experience" (Lewis, 1942), animals, denied verbal communications with man are unsuitable as test subjects on which to measure induced pain. Physiological accompaniments of pain (such as action potentials in nerve fibres and rise of blood pressure) and psychological accompaniments (such as apprehension), which themselves result in trails of physiological effects, are neither pain-experience, nor are they directly proportional to it. The only possibility therefore was to measure the pain following the administration of the noxious agents by allowing a human subject to describe his pain in terms of units
on a subjective pain-scale.

Such a scale was used by Wolff and his colleagues in the measurement of pain in man following the infliction of thermal stimuli (Hardy, Wolff & Goodell, 1947). In their experiments they employed a scale of ten and a half units (dols) representing twenty-one just noticeable differences in pain intensity above threshold. The scale used in the present experiments for the subject's description of his pain following the chemical stimuli has been a four unit one representing, above zero (= no pain), slight, moderate, severe, and very severe pain-intensities. Half units are employed and subjects find it convenient to even further subdivide and so to express their pain in 'quarter units'.

There is therefore a relationship between the 'just-noticeable-differences' of Wolff's subjects in their responses to thermal stimuli and the conveniently expressible differences in pain intensity in my subjects.

Description of pain tends to be inaccurate after the event. "One reason for inaccuracy and inadequacy of description is the difficulty of calling up exact memories of what has been felt some time previously. It is certain that the closer the description is to the event the more accurate it becomes, and that the description is most accurate when given at the time pain occurs". ....."Another reason is the difficulty of finding right words of description and apposite
illustrations; to do this requires observational and didactic skill which few possess" (Lewis, 1942). And so the simple 'four-unit' pain scale has been employed in these experiments concurrently with the applied pain-stimulus, and all the subject was required to do was to slide a freely-moving pointer over a short scale in accompaniment with the fluctuations in his pain.

The potential value of knowledge of time-intensity curves of pain-experience in the diagnosis and cause of pain was recognized by Lewis. "Pain may come and go in a flash, as when the skin of the face is pricked; it may rise to a plateau and last with little fluctuation for a long time before diminishing and vanishing as when the skin has been burnt or in an attack of angina pectoris; it may be felt in rhythmic pulses as in inflammation of dental pulp or in pulsating headache".....

"it may be experienced as longer and less rhythmic phases, as in intestinal colic" (Lewis, 1938). Such records with long-interval time-scales have since been used with patients in the study of analgesics (Keele, K.D., 1948). The making of graphic pictorial records by second to second observation of pain-experience, as has been done in the present experiments, is, however, a new and rewarding enterprise.

The pain-scale, and its use in the pictorial recording, was developed in conjunction with the developments in techniques of administration of the test-substances. It was essential that test-materials be
introduced to the body in a reliable and constant way which would allow of a comparative technique whereby an unknown could be compared with a known material as a standard of reference. The requirements of a suitable technique are:—(1) that the pain-responses to different concentrations of a pain-producing substance should be proportional to the concentrations administered; (2) that the administered substance, which may be toxic or infective, should be easily removable from the body.

Following the suggestion of Professor C.A. Keele to try the exposed base of a cantharidin-induced blister, solutions were applied to such a preparation and it was found to be satisfactory for effecting the comparisons since:—

1. The site of administration was constant throughout an experimental run.

2. A high degree of discrimination was obtained between Ringer-Locke solution and test-fluid administrations, and between various concentrations of test-solutions.

3. The great reluctance (born of experience) of subjects to submit to experiment was overcome as the blister-base method itself was much less unpleasant than injection procedures.

4. It was thought just permissible to apply material of doubtful suitability (e.g. infected) to willing subjects (mainly C.A.K.) for short periods and then to carefully wash off.
This technique of application of test-fluids to the exposed base of a blister for the testing of their pain-producing properties had never been thoroughly investigated or exploited. It has been found to allow the study of:

i. chemical excitation of pain;

ii. chemical antagonism of chemically induced pain;

iii. local chemical antagonism of mechanically induced pain.

(iii.) above was investigated by an experimental subject who adapted the technique to achieve a discriminating method for the study of local anaesthetics in man (Mongar, 1955).

The use of the blister-base technique in (i) and (ii), and the results so obtained using previously described agents and certain 'hitherto unknown' materials (such as the 5-hydroxytryptamine of blood clotting and the polypeptides of glass-exposed human plasma) are described in the thesis.
PART I.
PART I - METHODS.

1. INTRODUCTION.
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8. **Uses of the technique.**

C. **Conclusion.**
PART I. METHODS.

METHODS OF INTRODUCING CHEMICAL AGENTS INTO THE SKIN AND OF RECORDING PAIN AND ITCH.

1. INTRODUCTION.

The following methods of applying chemical excitants of pain to the skin have been used:

i. Pricking through a drop of solution.

ii. Intradermal injection.

iii. Application of solutions to the exposed base of a Cantharidin Blister.

2. RECORDING PAIN.

In early experiments the subject recorded his pain experience in handwriting. A numerical code was used:

0 = No pain.
1 = Slight pain.
2 = Moderate pain.
3 = Severe pain.
4 = Very severe pain. This was seldom encountered.

In addition, intermediate values (½, ¾ etc.) were used to denote intermediate intensities of pain. The maximum pain experienced in each fifteen seconds period was recorded. This mode of recording was soon rejected for two reasons:

i. It was quite impossible for the subject to
keep pace with rapid fluctuations in intensity of pain.

**ii. The records were unsuitable for immediate assessment** by inspection, let alone for the immediate comparison of results of several administrations. They were therefore unsuitable for accumulation for reference purposes. To improve on this method graphic recording of the pain experience was introduced.

**Graphic Recording of Pain.**

This provides immediately readable and readily stored records, suitable for subsequent reference. The results so obtained are, moreover, accurate second-to-second descriptions of the subject's pain experience.

The apparatus was set up as shown in Fig. 1. The writing lever was arranged to operate behind a pain scale so constructed that the subject should be unaware of the pain-picture he was making. The reason for this was to try to eliminate as far as possible the drawing of pain-pictures to preconceived visual conceptions. The use of the pain-scale already described (p. 25) is appropriate for the measurement of this subjective phenomenon.

The writing lever attached to a float on a mercury manometer was operated by means of
pressure changes transmitted to it through an air-containing rubber ball-and-tube system. The ball, B, was compressed or decompressed by turning the handle, H, to close and open clamp C in which the ball rested. Thus the subject traced the intensity and fluctuations of his pain on the smoked paper of the rotating drum as a graph with abscissa = time and ordinate = intensity.

Figure 2 shows pain-picture records so made. The intensity, time of onset, the rate of rise to peak, duration and rate of decay of pain can be seen at a glance. Such records which are quick to read are thus particularly suitable for large-scale investigations and for reference purposes.

Other subjective phenomena arising from local chemical administration.

1. Itch - provision was also made for the recording of itch in the same way as for pain. A subjective scale of two units was employed:

0 = No itch.
1 = Slight itch.
2 = Severe itch.

The levers recording pain and itch were aligned vertically on the drum so that the time courses of the two sensations could
be immediately compared (Fig. 34). The substances used in this investigation, with the exceptions of histamine and histamine liberators, rarely evoked any itching.

2. **Warmth.**

Other observers and myself have noted that when certain solutions were applied to the exposed base of a cantharidin blister, a sensation of heat varying from mild to considerable warmth was felt. This occurred either with low concentrations of pain producing agents (e.g. KCl, 5-hydroxytryptamine) on a sensitive area, or with higher concentrations on an area of lower sensitivity. The feeling of warmth is clearly neither itch nor pain and is not graphically recorded. Warmth occurs more frequently with substances which produce delayed pain (see below). It increases in intensity and frequently heralds the onset of pain itself, when the warmth sensation immediately disappears.

**Touch and Pressure.**

Sensations of touch and pressure have not been experienced after application of chemical agents to the cantharidin blister area.
Table 1 - 'PRICKING THROUGH'

Table showing maximum pain experienced after each application of 0.9% NaCl and 1%, $10^{-2}$ g/ml. ACh solutions, to three subjects.

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>DATE</th>
<th>0.9% NaCl</th>
<th>10^{-2} g/ml. ACh.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Individual Responses</td>
<td>Individual Responses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Pain</td>
<td>Total Pain</td>
</tr>
<tr>
<td>G.P.</td>
<td>19.10.51</td>
<td>0, ½</td>
<td>½, ½</td>
</tr>
<tr>
<td></td>
<td>9.11.51</td>
<td>0, 0</td>
<td>½, ½</td>
</tr>
<tr>
<td>J.W.M.</td>
<td>19.10.51</td>
<td>1, 1</td>
<td>½, 1</td>
</tr>
<tr>
<td></td>
<td>9.11.51</td>
<td>½, 1</td>
<td>½, 1</td>
</tr>
<tr>
<td>R.M.L.D.</td>
<td>19.10.51</td>
<td>½, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td></td>
<td>9.11.51</td>
<td>1½, ½</td>
<td>½, 1</td>
</tr>
</tbody>
</table>
3. **PRICKING THROUGH A DROP OF SOLUTION ON THE SKIN.**

**Procedure:**
A drop of the test-solution was placed on the cleaned skin of the flexor surface of the forearm. Four pricks were rapidly made through the drop with a No. 7 straight triangular needle so as to penetrate the epidermis without drawing blood. The subject then immediately started a stop-watch, \( t = 0 \), and wrote down the pain experienced during the following two minutes. The maximum pain felt during each fifteen seconds was recorded. Applications were made every five minutes on comparable areas on alternate forearms.

**Results.**
Table I shows the maximum pain experienced after each application of isotonic NaCl, \( 9 \times 10^{-3} \text{ g/ml.} \) (0.9% \( \text{w/v} \)) and ACh \( 10^{-2} \text{ g/ml.} \) to each of three subjects on two experimental days. It shows that the subjects could not distinguish between two substances applied in this way. They also failed to distinguish between isotonic NaCl, \( 9 \times 10^{-3} \text{ g/ml.} \) (0.9% \( \text{w/v} \)) and isotonic KCl solution, \( 1.15 \times 10^{-3} \text{ g/ml.} \) (1.15% \( \text{w/v} \)).

Histamine \( 10^{-6} \text{ g/ml.} \) produced itching as described by Lewis (Lewis, 1936a).

**Conclusion.**
In comparison with the other methods of testing to be described, this method is very insensitive. This is probably due to poor penetration of the applied solutions into the skin.

4. **INTRADERMAL INJECTION**

Procedure:

These administrations, like those of the "pricking through" technique, were made in comparable areas on the volar surface of alternate forearms. Fine needles, gauge 20 or 27 were used, and small volumes (0.05 ml.) of isotonic, neutral solutions were injected. The subjects were unaware of the time of injection of the solution as, firstly, they did not observe the operation and, secondly, there was a blank period of duration unknown to the subject, between the subsidence of the pain due to insertion of the needle and the injection of the fluid. This blank period, of minimum duration fifteen seconds, might be as long as three minutes, and was different for each administration. Sometimes, after injection of a solution producing no pain response, subjects would enquire whether the drug had been given. In any experiment in which the pain due to the presence of the needle did not subside, the needle was withdrawn and an entirely new administration was made elsewhere.
Table 2. Ringer-Locke Solution Administrations.

<table>
<thead>
<tr>
<th>METHOD OF APPLICATION of the R.I. Solution or 0.9% NaCl Solution</th>
<th>ADMINISTRATIONS</th>
<th>PAIN RESPONSE</th>
<th>SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of: -</td>
<td>Present $\frac{1}{2}$ on scale</td>
<td>Doubtful $\frac{1}{4}$ on scale</td>
</tr>
<tr>
<td>A. Cantharidin blister area technique.</td>
<td>111</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>B. Intra-dermal injection technique.</td>
<td>27</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>
Results.

Figure 2 (a) shows (i) pain due to insertion of the needle, (ii) the blank period of duration unknown to the subject, (iii) the pain due to injection of $0.05 \text{ ml. ACh } 10^{-4} \text{ g/ml}$. In view of the lack of penetration of the ACh in the "pricking through" technique, this was encouraging, but Figure 2b shows that an injection of $0.05 \text{ ml. of isotonic NaCl solution}$ can produce almost as much pain as $10^{-4} \text{ g/ml. ACh}$. The method was then used to provide data for statistical analysis.

Table 2 shows that if sufficient numbers of injections are made, statistically significant differences can be shown between the solutions used, viz., ACh $10^{-2} \text{ g/ml.}$ and isotonic NaCl. However, the labour involved and also the development of an emotional state in the subjects who actively disliked the many injections, necessitated the search for other methods of administration of test solutions. In Figure 3 (a and b) a typical experimental run using the injection technique is shown.

This may be compared with Figure 5 using the differentiating cantharidin blister area technique, to be described. In Figure 3 (a) some differentiation is apparent between isotonic NaCl and $10^2 \text{ g/ml. ACh}$ only on a
statistical examination or on "suitable" selection of results, whilst (b) two injections of ACh $10^{-5}$ g/ml., one thousandth strength of the former, have produced more pain than three injections of the $10^{-2}$ g/ml ACh solution.

Conclusion.
The intra-dermal injection technique is clearly an inferior method of experiment to the blister area method in which the intensity of pain produced is directly proportional to the concentration of the applied agent.

The lack of discrimination of the intra-dermal injection technique is most likely due to variation between administrations in their depth and position in relation to the tri-dimensional distribution of the pain nerve endings.

5. CANTHARIDIN BLISTER AREA TECHNIQUE.

A. Preparation of the Test-area.

A plaster about 1 cm. in diameter, containing 3 mg/g (0.3% w/w) cantharidin is applied to the skin of the flexor surface of the forearm for 6 - 8 hours during the evening before the day of the experiment. When the plaster is removed the area may show slight erythema, and during the night a blister develops of similar diameter to that of the plaster. Fig. 4 There is little or no sensation in the
treated area. The blister is opened on the following morning when, with the subject seated comfortably, his arm on an arm-rest in a room of temperature about 20°C. the blister is opened to reveal the blister base. Firstly the raised blister covering and the surrounding skin are cleaned with alcohol which is allowed to evaporate. The blister fluid is then aspirated (volume 0.07 to 0.2 ml.). The separated epidermis is cut away and the exposed blister base is bathed in a solution of the following composition:

<table>
<thead>
<tr>
<th>Solute</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na Cl</td>
<td>9 x 10^{-3}</td>
</tr>
<tr>
<td>K Cl</td>
<td>400 x 10^{-4}</td>
</tr>
<tr>
<td>Ca Cl₂</td>
<td>2.4 x 10^{-4}</td>
</tr>
<tr>
<td>Na HCO₃</td>
<td>1.5 x 10^{-4}</td>
</tr>
</tbody>
</table>

and called "Ringer-Locke Solution", R.L. During the manipulations just described, and for ten to fifteen minutes after the first application of the bathing fluid there is usually pain of slight to moderate intensity. After this there is usually no spontaneous pain.

When the area has become quiescent, and before the application of solutions to be tested
for pain producing activity, the bathing fluid is removed and the area is dried with filter paper. The test solutions, isotonic with blood and of pH 6 - 7.5 except where the effects of pH and tonicity were themselves being investigated, are warmed to about 30°C before application in volume of about 0.2 ml. The solutions are applied so as to cover the whole of the area as quickly as possible, then left in contact for one or two minutes unless it is specifically desired to examine the effects of more prolonged application. (As a rule it is best to avoid protracted responses and long experimental sessions which are liable to cause fatigue). The applications of test solutions are made in a constant way, and in such circumstances that the subject gets no clues as to the nature or concentration of the substance used. In a few experiments the nature of the solution was also unknown to the person who made the applications. This precaution was found to be unnecessary since the effects elicited by the test solutions are usually so clear-cut as not to be susceptible to suggestion.

After application the solution is removed with a pipette and the area washed and covered with
the bathing fluid. The usual interval between applications of test solutions is ten minutes, which is sufficient to allow the sensitivity of the area to return to normal. Tryptamines, local anaesthetics and certain other substances may produce longer refractory periods. Up to ten or twelve applications may be made in one experimental session without causing fatigue. The area remains sensitive for up to two days and sometimes forty to fifty applications have been made on a given blister area during this period.

At the end of the experiment, proflavine sulphate solution, $10^{-3} \text{g/ml.}$ is applied to the blister area and the area is then allowed to scab over without any dressing. There have been no "infections" during the course of four hundred and three experiments on one hundred and six blister areas in twenty-eight subjects (twenty-six Male and two Female) and it has not been found necessary to use bacteriologically sterile solutions in this work.

B. The Area in Action

1. Graded responses to acetylcholine administration.

Fig. 5 (a) and (b) shows pain responses
in two subjects, (D.A. and J.W.M.) to the application of different concentrations of ACh. It is not at all uncommon to find subjects discriminating easily between ACh $10^{-4}$, $5 \times 10^{-5}$, $2.5 \times 10^{-5}$ and $10^{-5}$ g/ml. and R.L. solution. Both the records also show completely negative responses. In (a) the blank is to ACh after a suitable antagonist (atropine, see under Part I, Methods, and under Part II, acetylcholine - atropine), in (b) to plasma.

Thus one can induce pain of at least four different intensities by administration of suitable concentrations, each for each, within the range $10^{-5}$ to $10^{-4}$ g/ml. ACh.

This test preparation, i.e. the subject and his test area, used in this way, is therefore, a sensitive and discriminating one for use in the comparison of pain-producing solutions.

2. Absence of response to applied Ringer Locke solution.

Table 2 (A) shows the results of applications of R.L. in ninety-two experimental sessions on sixty-two blister areas in eleven subjects. It will be seen that in one hundred and eleven administrations none produced pain of greater intensity
than \( \frac{1}{3} \) unit, ninety-two produced no pain at all and the remaining fifteen caused pain of less than \( \frac{1}{3} \) unit intensity. In addition we have frequently observed no pain responses to small concentrations of substances which in higher concentrations produced definite pain. In all these experiments the subject discriminated clearly between R.I. and \( 10^{-5} - 10^{-4} \) g/ml. ACh.

It is thus clear that whenever the intensity of pain exceeds \( \frac{1}{3} \) unit the observation is significant. Pain of \( \frac{1}{3} \) unit is also significant when it is recorded during any given experiment in which there is no response to bathing fluid or to subliminal concentrations of pain-producing substances. Similar grading of response occurs with the other pain-producing agents and, moreover, the significance of pain of \( \frac{1}{3} \) unit intensity increases as the sensitivity and reliability of the subject grow with experience. The tracings of Fig. 6 (a) and (b) were made by two experienced subjects, and show examples of the reliable pain differentiation possible by this technique. In the (a) tracing, subject J.W.M. has clearly distinguished between the effects of \( 10^{-5} \) g/ml.
of one substance, Ach, which produced no pain and $10^{-8}$ g/ml of another, 5-HT, which caused pain estimated as just over one unit intensity. In the (b) tracing the subject, (R.M.L.D.), has distinguished between R.I. and $10^{-8}$ g/ml. tryptamine, both of which were non pain-producing, and $10^{-8}$ g/ml. 5-hydroxytryptamine which elicited pain of $\frac{3}{4}$ unit and was therefore estimated as being significant. Pain production by $10^{-8}$ g/ml. 5-HT has been subsequently confirmed in other subjects, as has also the greater pain producing potency of the 5-hydroxyl compound, 5-HT, over unsubstituted tryptamine (see Part II, 5A).

Compare the results of twenty-seven administrations of isotonic NaCl solution in eight experimental sessions in four subjects by the intra-dermal injection technique - Table 2 (B). There, only ten out of twenty-seven responses were completely negative as compared with ninety-two out of one hundred and eleven in the cantharidin blister area experiments.

Following injections most responses are of $\frac{1}{2}$ unit and greater intensity, whereas, with the C.B.A. technique the reverse is overwhelmingly the case and no response over
3. **Duration of accurate discrimination.**

The period during which the various concentrations may be accurately detected by the subject may be up to one and a half hours or longer during an experimental run provided that no interfering substance has been administered in adequate concentration. Fig. 7 shows the results of applications of ACh, R.L. and subthreshold concentrations of two materials designated "A" and "B", during a session of eighty minutes. It provides in addition, yet another example of the lack of force of suggestion and of mechanically induced pain with this technique of application.

The area remains sensitive for up to two days and three sessions as described, or more shorter ones, may be performed each day. The method is comparative and on experiment usually consists of an experimental run which may be upwards of two applications where one administration is a control or reference solution.

4. **Factors influencing discrimination by a subject between different Concentrations of a given solution.**

i. **Frequency of Administration.**
Experimenting with $5 \times 10^{-4}$ g/ml. ACh solution it was found that when the time-interval between applications was $\frac{1}{2}$, 1, 2 or 4 minutes the pain responses obtained were far below the intensity following the first application. When the interval between applications was nine minutes the pain intensity was the same on each occasion. Fig. 8

Similarly for KCl, another pain-producing agent, in $2/3$ isotonic concentration ($7.7 \times 10^{-3}$ g/ml., 0.77% w/v). Little response occurred when administrations were as close as 10 sec., 2 min. and 5 min. to one another, whereas a rest interval of 9 minutes once more allowed the area to recover so as to give the original intensity of pain. Fig. 9

Thus I have used, and found to be generally satisfactory a resting period of nine minutes between applications; the solutions themselves being applied for periods of $\frac{1}{2}$ - 2 minutes, sometimes longer, according to the investigation under study.

Occasionally a longer refractory period is induced by a chemical e.g. by 5-hydroxy-tryptamine, and by serum. An illustration of this is in Fig. 10 where the responses
to both 5-HT and to serum are seen to decrease progressively. When the response
to 10 times the original dose of 5-HT has become less than the first response,
the response to ACh is little changed.

The Use of Acetylcholine as an Indicator of the Pain Sensitivity of a Cantharidin
Blister Area.
Where one substance is under investigation a control standard of the same material
may be used intermittently to determine the responsiveness of the area and to measure the potency of the test solution (just as in objective observations on completely isolated organ systems.) When the chemical nature of the test solution is not known, or when several different agents are to be applied in one experimental run, it is useful to test the sensitivity of the area by applications of acetylcholine. In the course of four hundred and three experiments on one hundred and fifty-six blister areas, (twenty-eight subjects - twenty-six Male, two Female) different concentrations of acetylcholine have been applied as a means of estimating the sensitivity of each individual area and the reliability of the subject during each
experiment.

It has usually been found that when the pain responses to acetylcholine have been of a low grade (e.g. 4 unit for $10^{-4}$g/ml. Ach) the responses to other markedly painful chemical stimuli (e.g. distilled water, isotonic KCl) have also been low. In these circumstances I have often noticed that these poor responses may be due to:

a. a **film of exudate** forming over the blister area,
b. **fatigue of the subject** setting in following numerous administrations, or
c. **local inhibition** of the area resulting from an administration of an inhibiting chemical or a high concentration of a usually non-inhibiting one (i.e. non-depressant after the lapse of the usual 9 min. rest interval).

When the area is insensitive to Ach, it is usually insensitive to other chemicals. However, substances which differ from Ach in mode or site of action can produce responses which are not in proportion to those produced by Ach.

In agreement with a basic principle of bioassay, Ach cannot be used as a standard for the assay of the pain-producing actions
of other chemicals.

ii. Rate of Application of Solutions to the Cantharidin Blister Base.

Fig. 11 (b) shows variation in pain responses resulting from slow and fast administrations of a given concentration $10^{-3}$g/ml.) of ACh, whilst the initial part Fig.11(a) shows similar variation in response but caused by change in concentration administered.

It is obvious that to obtain discrimination solutions must be applied rapidly to the blister base - slow administration results in poor discrimination. The reason for this may be that the blister-base preparation is measuring rate of change of its environment.

iii. Size of the Blister Area.

The size of a reformed blister area was reduced from total to one quarter by application of low melting-point ($45^\circ$) paraffin wax to the area. This is not a painful procedure and the wax may be removed and the whole area used, once more, during the same experimental session.

Fig. 12 shows the effect of change of area size on the pain response to ACh. On the $\frac{1}{4}$ blister area pain was of low intensity
and no discrimination was observed between administrations of Ach at concentrations of $5 \times 10^{-5}$ and $10^{-3} \text{g/ml}$. On the large area, not only was the pain more intense but discrimination was possible even between $10^{-5}$, $5 \times 10^{-5}$ and $10^{-3} \text{ g/ml}$ of Ach.

iv. Conclusion

To achieve accurate discrimination between pain-producing solutions account must be taken of:

i. Frequency of administration

The most convenient interval between administrations is 10 min.

ii. Rate of administration

This should be as rapid as possible to the whole area.

iii. Size of blister area

The diameter of the area should be not less than 0.75 cm.

iv. Sensitivity of blister area to the test substance and the use of Ach as an index of sensitivity.

v. Duration of experimental session

This should not exceed 1½ to 2 hours.

5. Features of the Pain Response.

i. Typical Pain Responses to Ach, K$^+$ and to 5-HT.
The typical pain-response to acetylcholine application.

Acetylcholine induced pain is of immediate onset, rises at once to a sharp needle-like peak, and then declines very rapidly. Sometimes, as the pain subsides, there are a few "after-discharges," (Fig. 7). Occasionally a sensation of slight itch, occurs, (Fig. 7). The whole response may last up to 30 - 40 seconds. This typical action is found following the application of any one of a wide range of concentrations from those eliciting severe pain to those which give responses of only ½ unit intensity.

The pain-responses due to a number of other substances differ from that induced by acetylcholine in the following respects:

a. The range of concentrations over which the time of onset of pain is immediate, or almost so. Threshold concentrations of ACh result in a pain response after an interval of from 0 - 2 sec., higher concentrations give immediate pain - Fig. 13 (a) i, Fig. 13 (b) i.

Though high concentrations of K⁺ may give immediate pain, lower concentrations produce pain only after some delay
Similarly, high concentrations of 5-HT can produce intense pain of immediate onset but concentrations giving moderate or slight pain do so only after an interval of 10 - 45 secs. - Fig. 13 (b) ii.

b. Time taken to reach peak response for the given dose.

Once initiated the ACh pain rises immediately, or almost so, to its peak. This is not the case with concentrations of K+, or 5-HT giving moderate responses. These take several seconds to reach their summits or rather plateaux.

Compare Fig. 13 (a) response (i), ACh, with that of (ii), KCl, and Fig. 13 (b) response (i), ACh, with that of (ii), 5-HT.

c. Duration of response

The ACh response is needle-like over a wide range of concentrations and the whole is frequently over by 10 - 15 secs. This is unlike the long lasting effects of K+, 5-HT, and of hypo and hypertonic solutions, which may continue for from one to several minutes. (Fig. 13(a) and (b) Fig. 21 (a) and (b).

There is usually a relationship
between total duration of response and concentration of ACh applied in an experimental session eg. low concentrations usually produce responses lasting from 15 - 20 sec. whereas the effects of high concentrations often last during 15 - 45 sec.

ii. Variations

a. Variations between subjects.

The same subject produces the same response picture to ACh application from session to session and also from blister to blister.

Different subjects usually produce different pain-response pictures whilst still conforming to the typical ACh response-pattern as already described. The differences lie chiefly in the subsidence of the pain. Tracings made by any one subject have individual characteristics and can be readily identified in a collection of records from several subjects. The responses tend to be of two main types, as exemplified by the responses of J.W.M. and R.M.L.D. respectively - Fig. 6 (a) and (b). For example the responses of subjects W. and C.A.K. for
acetylcholine (Fig. 14 (a) and (b)) resemble those of subject J.W.M. (Fig. (b)), whilst the responses of the author, D.A., are intermediate between the two types (Fig. 5 (a)). Likewise other substances produce characteristic pain-pictures, which are readily distinguishable in spite of individual variations in response. The occasional occurrence of itch as the pain due to ACh subsides does not belong to any one reaction pattern. It takes place more frequently with some subjects than with others. See Fig. 7, where the upper tracing is the itch record.

b. Effects of first administrations.

The first administration of ACh to a cantharidin blister area made about ten minutes after exposure of the blister base, and after subsidence of any spontaneous pain, frequently results in an atypical pain response. The onset of pain may be delayed for two or three seconds, the pain may take two or three seconds to reach its maximum, which itself is prolonged, and the whole pain lasts longer than is usual for ACh. (Fig. 14 (a) and (b)).
The effects cannot readily be attributed to the effects being threshold ones for pains of 1 and 2 units intensity, as shown in the figure, are well above average threshold intensity pain for ACh (concentrations of ACh to give pain responses of \( \frac{1}{2} \) unit intensity and less can usually be found). Fig. 14 (a) shows the second response of a series giving the typical needle-like ACh response though it was induced by only half the concentration of ACh to that used for the first and protracted administration.

Fig. 14 (b) shows the response to the same concentration given as both the first and third administrations of a series. These atypical responses are seldom seen after the first administration itself. They are not due to the effects of the blister fluid which had, till recently, bathed the areas, since the application of blister fluid to the area does not so change the contours of the pain response to subsequent applications of ACh.

c. **Low sensitivity - film formation.**

From time to time a coagulum forms quite rapidly over the base of the blister,
even whilst it is bathed in R.L. fluid, and access of chemical excitants of pain is prevented. Removal of the film (this is sometimes very painful) restores the sensitivity of the area to more normal values. The responses are then usually well maintained unless exudation continues.

Fig. 15 shows the effect of removal of a film which had formed in the course of an experiment on subject C.A.K. The order of sensitivity then achieved remained during the rest of the day.

6. Structures acted upon by the applied Solutions

1. The Blister and its Base - Structure.

I am greatly indebted to Dr. Jeffrey Boss, of the Middlesex Hospital Medical School, for subjecting himself to biopsy and for his work on the histological appearances of that part of the skin remaining after removal of the top of a cantharidin blister. He found that the tops consisted of stratum corneum and stratum lucidum and contained no nerve fibres, and that "the outermost remaining layer of the skin, apart from cells free in the blister fluid, is the stratum basale of the epidermis. This layer is for the most part complete,
but the bridges connecting its cells laterally are no longer present, the intercellular spaces are abnormally wide, and the histological appearances suggest that a solute in fluid applied to the exposed base of the blister could easily reach the derma between the basal cells. Fine nerve fibres, associated in the literature with the sensation of pain, can, in normal skin, be traced from the derma between the cells of the stratum germinativum. It is not known whether these fibres are damaged as the rete Malpighi disintegrates around them, but, whether damaged or intact, they probably lie free in the blister cavity, and are immediately bathed by any fluid placed upon the base of an opened blister. The penetration of substances in this fluid to endings in the derma is also possible."


It is interesting to note the actions of ACh and $K^+$ on these exposed fine nerve fibres and endings of the blister base. $K^+$ like ACh is a pain stimulant when applied to the exposed base. Here, as on other nerves, it can apparently
stimulate nerve fibres for it will stimulate during the period of refractoriness to ACh (an agent presumed to act at nerve terminals)—Fig. 16 ACh, on the other hand will not act during the period of refractoriness to K⁺ administration, Fig. 17.

It thus appears that substances administered to the exposed base of a cantharidin blister can act on both nerve endings and fibres.

iii. The Relationship of Cantharidin to the Effects produced.

a. Actions of cantharidin on the blister area

Cantharidin itself, 1 mg/ml. in arachis or olive oil did not produce pain during two minutes contact.

b. Actions of cantharidin blister fluid and burn blister fluid on the cantharidin blister area.

The fluid aspirated into a glass syringe from the cantharidin blister and applied to the cantharidin blister base a few minutes later, was found to be pain-producing and to have time-action characteristics very different from those of other substances examined up to that time (viz. ACh, KCl, hypo and
hypertonic solutions and acids.

Fig. 18 (a). Fluid aspirated from a burn blister was found to behave exactly similarly on the cantharidin blister area, Fig. 18 (b), (as also did cantharidin blister fluid and burn blister fluid when applied to the exposed base of a blister raised by heat.)

o. Actions of chemicals on cantharidin and burn blister areas.

Burn blister areas were produced on the forearm by application of a metal disc heated in boiling water and quickly applied and left in contact with the skin for 1 - 3 seconds. When blisters developed they did so after a delay of 15 - 24 hours.

ACh applied to these exposed areas gave its typical sharp pain effects, graded according to concentration of ACh employed. Low concentrations of chemicals and of R.L. were painless and applications of other chemicals e.g. 5 - HT, B.B.F., or C.B.F. to these burn blister bases produced the same pain pictures as they do on the bases of cantharidin blisters. Figure 19 shows the effects of R.L., 5 - HT and ACh
applied to the respective bases of cantharidin and heat blisters. There is no evidence that cantharidin produces an abnormally sensitive state in the exposed blister base. It was therefore felt that results obtained by this method of study would also apply to other types of injury to the skin.

7. **pH** and **Tonicity Effects**.

i. **Alkaline and Acid Solutions**

Alkaline solutions have not been found to be pain-producing (pH's 7 - 9). Highly acid solutions e.g. HCl pH 2 are markedly pain-producing. Between these two there is a wide range of pH's in which no pain is felt. There is a sharp end-point between a pH which will just not cause pain and one that will. It may be as little as 0.5 pH unit or less. The pain when it does appear is sharp and unmistakable. Responses to HCl and to HCl and lactic acid, (by subjects C.A.K. and D.A. respectively,) are shown in Fig. 20 (a) and (b). Tracing (c)(C.A.K.) of the same figure shows absence of significant pain from administration of citric acid at pH's 6.5 and 3.7 but pain
of 2 units intensity when citric acid pH 3.1 was applied.
In general pain due to pH may be expected from solutions of pH 3 and below. Pain production by solutions of pH 5 and above is due to some factor other than the hydrogen ion concentration of the fluid applied.

ii. Effect of pH on Pain induced by ACh Solutions.
Not only is there a wide range of pH's within which pain does not occur, viz. 4 - 9, but also the pH of a solution of ACh does not markedly affect the intensity of pain produced by it. This was shown by administrations of ACh buffer solution at pH's between 4 and 8.

iii. Tonicity.
Just as there is a wide range of pH's which do not cause pain so there is a wide range of tonicities within which pain does not occur.
Distilled water and \(5 \times 10^2\) g/ml (5% w/v) NaCl are both markedly painful when applied to the exposed blister-base. (Indeed the painful effects of tap water and common salt on cuts are common knowledge). The threshold for pain effects due to tonicity
are X 1/3 and X3 isotonic. Fig. 21 shows the marked effects of distilled water and of \(5 \times 10^{-2}\) g/ml. (5% w/v) NaCl i.e. X5 isotonic as compared with the absence of pain on application of isotonic R.I. - tracings (a) and (b) respectively.

iv. Conclusion

In conclusion it may be said that small divergencies from isotonicity or from pH 7 do not interfere with the pain-producing properties of solutions applied to the blister area.

8. Uses of the Technique

Using the blister area method as described one may:

1. Detect substances which produce pain when administered in this way,

2. Determine their effective concentration,

3. Study the relationships between chemical structure and pain-producing action (see under ACh and 5-HT below pp. 62 and 85).

4. Identify the effective agent in a pain-producing solution by relating the effects to those of chemicals whose action is known. For example, the type of pain produced by agents applied to the exposed base of a cantharidin blister falls into two distinct categories:
a. that of immediate onset e.g. ACh, acidity, hypotonicity,
b. that of delayed onset e.g. 5-HT, blister fluids and certain polypeptides.
In category a. the delay is usually under 1½ sec. and in category b. the delay is usually over 15 sec. and is frequently between 25 and 45 sec. The actions of K⁺ fall intermediately with delay periods of 2 - 7 secs.
Furthermore, certain agents make the area increasingly refractory to themselves, e.g. the tryptamines, whilst the increase in refractoriness to other materials is much less marked. This provides two further means of identification:

i. Production of refractoriness - whether or not it occurs due to the unknown agent.

ii. The specificity of the refractoriness produced.
The latent period and refractoriness of solutions should be compared with those of selected standard solutions in blister area tests.

5. Study antagonists to pain production.
Pain in the blister area has been produced experimentally by chemical or mechanical
a. Chemically induced pain.

The use of the area in the study of chemically induced pain is illustrated in Figures 22, cocaine, and 23, nicotine, where antagonism by these agents to $6 \times 10^{-5} \text{g/ml}$. ACh-induced pain is shown. The pain response to the acetylcholine was initially determined and the area then bathed for ten minutes in the supposed antagonist. The nature of the bathing was unknown to the subject (the solution being applied in the usual manner for R.L. bathing between doses) as was the nature and potency of the applications of pain-producing chemical. The antagonistic bathing fluid was blotted off in the usual way and a chemical stimulant applied. Subsequent bathings were with R.L. solution. The graphs show the immediate (depressing) effect on the ACh responses caused by the bathing in the antagonist and the recovery of the tissue after removal of the antagonist. Each figure shows the effect of bathing the area with two different concentrations of antagonist.
The depressant effects produced were proportional to the respective concentrations of antagonist used. With cocaine the higher concentration produced the greater and more prolonged depression of ACh responses. With nicotine, both concentrations used reduced the pain due to ACh to zero, and recovery after the higher concentration was very greatly delayed as compared with the rapid recovery after the bathing in the lower concentration.

b. Mechanically induced pain

The pain-sensitive cantharidin blister base has been used for the testing of local anaesthetics against mechanically induced pain by Dr. J.L. Mongar of University College, London. His method involves random distribution of the administrations of local anaesthetic solutions and of R.L. bathing fluid to the exposed base in a "blind" (2 x 2) or (3 x 3) assay, whilst the area is pain-stimulated with a weight loaded brush. In terms of the number of subjects required for an assay of given accuracy, Mongar's blister-base technique is about 20 times more efficient than the intra-
dermal weal method of testing local anaesthetics. (Mongar, 1955).

C. Conclusion.
The cantharidin blister area is a sensitive and discriminating preparation whereby one may study, chemical aspects of excitation and antagonism of cutaneous pain induced by chemical or other means.
The responses represent the subjective effects of chemicals acting on the exposed fine nerves and endings of the basal layer of the epidermis. The cantharidin does not create an abnormally sensitive state.
Different subjects give comparable results, as does the same subject on different days with different blister areas.
PART II. - PAIN PRODUCTION BY CERTAIN DESCRIBED SUBSTANCES OF TISSUE ORIGIN AND RELATED CHEMICALS.

1. ACETYLCOLINE.
   A. Pain-producing Actions of Acetylcholine.
   B. Relationship of chemical Structure to Pain-production.
   C. Antagonism of acetylcholine-induced Pain.
   D. Neostigmine.
   E. In conclusion - Discussion.

2. POTASSIUM ION.

3. SYMPATHOMIMETIC AMINES.

4. HISTAMINE
   A. Applied Histamine.
   B. Liberated Histamine.

5. INDOLEALKYLAMINES.
   A. A Study of the Actions of various Amines.
   B. Antagonism of tryptamine-induced Pain.
   C. Conclusion.

6. ADENOSINE TRIPHOSPHATE.

7. HYDROCHLORIC, LACTIC AND CITRIC ACIDS.

8. POSTERIOR ROOT EXTRACT.

9. CERTAIN POLYPEPTIDES.

10. CONCLUSION.
1. ACETYLCHOLINE

A. The pain-producing Actions of Acetylcholine.

Solutions of acetylcholine cause pain when applied to the exposed base of a cantharidin blister in concentrations down to about $5 \times 10^{-6} \text{g/ml}$. In early work ACh showed the usefulness of the blister area for studying chemical production of pain. Now, after hundreds of experimental sessions on many blister areas it is still used to reveal the pain sensitivity of an experimental area. I have described (p. 45) the typical needle-like character of the acetylcholine induced pain and the refractoriness which follows it, (p. 39). This refractoriness is not cumulative even with high concentrations of ACh (eg. $5 \times 10^{-4} \text{g/ml}$.) when the nine minute rest interval between doses is used.

It would appear that the action of acetylcholine is on the pain nerve origins rather than on the nerve fibres proper, since $K^+$ will stimulate pain during the refractory period induced by ACh (p. 51).

B. Relationship of Chemical Structure to Pain production - Study of the Actions of:

1. acetylcholine.

2. carbachol.

3. methacholine.
4. pilocarpine.

5. nicotine.

1. Acetylcholine - see pp. 45-62

2. Carbachol

Figure 24 (a) and (b), (subject B.C.) shows that carbachol can cause pain like that produced by ACh. This figure, together with Fig. 25 (D.A.) show that carbachol is one tenth to one twenty-fifth as potent as ACh and that the smallness of the pain response is not due to refractoriness produced by previous applications of ACh.

3. Methacholine. (Acetyl-B-methyl choline)

Unlike acetylcholine, acetyl B methyl choline is not pain-producing. Figure 26(a) and (b) shows absence of pain response to acetyl B methyl choline, M, in concentration of $10^{-3} \text{g/ml.}$ in two subjects (D.A. and C.A.K.) when acetylcholine $5 \times 10^{-5} \text{g/ml.}$ elicited pain of 2-3 units intensity.

4. Pilocarpine

In Fig. 26 (b) it is shown that pilocarpine, P, is, like methacholine, also inactive at a concentration of $10^{-3} \text{g/ml.}$ (pilocarpine nitrate) although the blister area is fully sensitive to ACh.

5. Nicotine tartrate

Application of nicotine tartrate, to the exposed
base of a cantharidin blister produced pain in each of the four subjects on whom it was tested. The concentrations used ranged from $10^{-4}$ to $5 \times 10^4 \text{g/ml.}$ and the intensity of the pain produced ranged from 1 to $2\frac{1}{2}$ units. The pain-production is not due to the tartrate fraction as shown in Fig. 26b where prior administration of sodium tartrate, $10^{-3} \text{g/ml.}$, elicited no pain, though the subsequent administration of a much lower concentration of active principle produced marked pain. Following the administration of nicotine, the pain-response to the application of ACh is reduced. Fig. 26b shows that pain of 2 units intensity elicited by ACh $5 \times 10^{-5} \text{g/ml.}$ was reduced to 1 unit intensity at ten minutes after nicotine tartrate ($5 \times 10^{-4} \text{g/ml.}$) had acted on the area for one minute only. After a further ten minutes the sensitivity of the area to ACh had recovered. When, however, the nicotine solution is applied to the blister base in place of bathing fluid, for ten minutes, the pain responses to ACh can be completely abolished. Fig. 23, presented, p. 58, to illustrate the use of the blister area in the study of pain antagonists, is a graph showing the effects, in two respective experiments on the same test-area, of different concentrations of nicotine tartrate,
viz. $10^{-4}$ and $5 \times 10^{-4}$ g/ml., applied for ten minutes, on the pain induced by acetylcholine. Responses are shown to application of $6 \times 10^{-5}$ g/ml. ACh immediately before, and at various intervals after the application of nicotine tartrate. In each experiment the nicotine itself, when applied produced pain (not shown), and the ACh response was reduced in each case from about $1\frac{3}{4}$ units to zero. The graph shows the higher concentration of nicotine (nicotine tartrate, $5 \times 10^{-4}$ g/ml.) produced a longer lasting depression than the lower concentration (nicotine tartrate, $10^{-4}$ g/ml.) the durations being 30 - 60 min. for the higher as against 5 - 10 minutes for the concentration of antagonist one-fifth the strength.

C. Antagonism of acetylcholine-induced Pain -

Actions of:

1. i. nicotine
   ii. d-tubocurarine
   iii. decamethonium
   iv. hexamethonium

2. Atropine

3. Various local anaesthetics e.g.
   i. cocaine.
   ii. procaine.
   iii. cinchocaine.
i. Nicotine - see page 63 et seq.

ii. d-tubocurarine

Figure 27 shows the antagonistic action of d-tubocurarine chloride, $10^5$ g/ml, on the pain response to ACh $10^{-4}$ g/ml. in subject R.M.L.D. The tubocurarine, applied after an administration of ACh, $10^{-4}$ g/ml., was left on the area for ten minutes. The area was then dried and the ACh $10^{-4}$ g/ml. was re-applied within one minute of removal of the antagonist. It did not cause pain.

Evidence of recovery of the area was seen after a further ten minutes, and by twenty minutes almost complete recovery had taken place.

iii. Decamethonium

Decamethonium iodide was almost as active as d-tubocurarine in blocking the pain-producing action of ACh. Unlike nicotine, though like succinylcholine, decamethonium did not cause pain when applied in a concentration of $10^3$ g/ml.

Figure 28, C.A.K., shows that the blocking actions of decamethonium iodide on the responses of the blister area to acetylcholine are pronounced.

The application of decamethonium iodide $10^{-5}$ g/ml as bathing fluid for eight minutes, reduced
the pain-response to $10^{-4}$g/ml. Ach (2 units intensity pain) to that elicited by half the concentration of Ach, $5 \times 10^{-5}$g/ml., (½ unit intensity pain). Following the administration of R.L. once more as bathing fluid recovery was almost complete at the time of the first subsequent administration. A higher concentration of decamethonium iodide, viz. $10^{-3}$g/ml., produced complete and long-lasting block of the Ach responses; even at 18 minutes after washing off the decamethonium recovery was still incomplete as shown by the low response to $10^{-3}$g/ml. Ach.

iv. Hexamethonium

In contradistinction to the effective blocking of the pain-response to acetylcholine by d-tubocurarine and by decamethonium hexamethonium is a relatively poor blocking agent. Figures 28 and 29 show that hexamethonium bromide $10^{-4}$g/ml. applied as bathing fluid between applications of $10^{-4}$g/ml. Ach produced very little depression of the Ach response. In Fig. 28 the ineffective concentration of hexamethonium used (viz. $10^{-4}$g/ml.) was ten times greater than that of the decamethonium used in the same experiment and which produced complete depression of Ach-induced pain. A concentration of $10^{-3}$g/ml. hexamethonium applied similarly, (Fig. 29) depressed the
pain-response elicited by the ACh from 'severe' intensity (13 units), to zero, and recovery of sensitivity to ACh was rapid (by 10 min.). The hexamethonium itself produced no pain.

2. **Atropine.**

   Figure 5 (a), shows the blocking action of atropine sulphate $10^{-4} \text{g/ml.}$ on the pain responses to acetylcholine. In the figure it is shown that bathing in atropine has rendered the blister area insensitive to acetylcholine $2.5 \times 10^{-5} \text{g/ml.}$ thus reducing the response to ACh to less than that elicited by $10^{-5} \text{g/ml.}$ ACh before the atropine was given. Recovery of the area was complete at the time of the next application of ACh. The block produced by atropine is apparently greater than that by hexamethonium though somewhat less than that elicited by d-tubocurarine, or by decamethonium.

3. **Various local anaesthetics** e.g.

   i. cocaine
   
   ii. procaine
   
   iii. cinchocaine

   The solutions of local anaesthetics used in this section were made just alkaline ($\text{pH } 7.5$) by addition of $\text{NaHCO}_3$.

   1. **Cocaine**

   The relative depressant action of two concentrations of cocaine hydrochloride on
the pain response to a given concentration of ACh was shown, Fig. 22, to illustrate uses of the cantharidin blister area technique in the study of pain antagonists, p. 58. In the figure cocaine hydrochloride applied as bathing fluid for ten minutes is shown to antagonise 6 x 10^{-5} g/ml. ACh, 5 x 10^{-4} g/ml. cocaine causing partial antagonism and 10^{-3} g/ml. complete antagonism of the ACh response.

11. Procaine.

Figure 30 (a) shows the antagonism to acetylcholine induced by procaine hydrochloride (10^{-4} g/ml.). After 10 mins. bathing with procaine no response followed the application of 5 x 10^{-4} g/ml. ACh, a concentration which had previously caused 'severe' pain (3½ units). After a further two minutes bathing, 10^{-3} g/ml. ACh caused slight to moderate pain (1½ units) and, after a further five minutes exposure to procaine, 10^{-2} g/ml. ACh caused only slight pain (1 unit). When bathing in R.L. was resumed the area recovered as is shown in the next two responses which are to the 5 x 10^{-4} g/ml. ACh. Full recovery was present at twenty minutes after removal of the procaine.
iii. Cinchocaine.

The powerful antagonistic action of cinchocaine to ACh induced pain is illustrated by the responses of subject J.L.M. in Fig. 30b. Though bathing in cinchocaine hydrochloride $10^{-5}$ g/ml. had little effect on the pain due to $5 \times 10^{-5}$ g/ml. ACh, bathing in $10^{-4}$ g/ml. cinchocaine reduced the pain due to $5 \times 10^{-5}$ g/ml. ACh to zero, after which five and even twenty, times that concentration failed to produce pain. Two hundred times the original concentration of ACh, viz $10^{-2}$ g/ml., then induced the original intensity of pain, 2 units. The response was delayed and atypical. No recovery of response to ACh $5 \times 10^{-5}$ g/ml., or even to $10^{-3}$ g/ml., was observed after bathing the area in R.L. solution for 10 minutes, but after a further 10 minutes of bathing, making twenty minutes in all the responsiveness of the area to the original low concentration of acetylcholine — $5 \times 10^{-5}$ g/ml. was almost completely restored.

D. Neostigmine.

No potentiation of acetylcholine action has been observed following bathing of the test-area in neostigmine. Figure 31 shows responses to
ACh 3 x 10^{-5} g/ml. and 10^{-5} g/ml. administered alternately (responses 1 to 4) after which, and before each administration 5 and 6 the area was bathed in neostigmine sulphate 10^{-4} g/ml. for 8 minutes. No potentiation of the responses to the administrations of the low concentration of ACh (10^{-5} g/ml.) occurred. If self suggestion were operating, the subject might well have been expected to draw large pain responses but this did not happen. That the area was not 'deteriorating' is shown by the last administration, 7, that of 3 x 10^{-5} g/ml. ACh which caused just as much pain as it did following earlier administrations (responses 1 and 3.)

E. In Conclusion - Discussion.

The actions of acetylcholine on the cantharidin blister area have been studied in order to show:
1. the responsive state of the blister area.
2. the properties of the receptors stimulated, and
3. the possible rôle of acetylcholine in the production of pain in the skin.

1. The responsive State of the Blister Area.
   This has been fully dealt with - p. 41 et seq.

   Certain properties of the pain receptors of the cantharidin blister area have been revealed by bathing the area in various pharmacological agents. The experiments employing K+ showed
that ACh acts on the pain nerve endings rather than on the fibres and is in accord with observations of Brown and MacIntosh (1939) and Brown and Gray (1947).

The immediate pain response elicited by ACh suggests that its action is a direct one on the nerve endings. The fact that carbachol also produces pain of the same type as that produced by ACh, whereas methacholine and pilocarpine are relatively inactive, suggests that the stimulation is "nicotinic" in type. The idea is corroborated by the actions of nicotine itself which first of all stimulates the area, causing pain, and then produces a prolonged depression of the area to ACh and to itself.

The question arises, have these pain receptors ganglionic or motor end-plate properties or, on the other hand, have they no particular resemblance to either?

Low concentrations of d-tubocurarine and decamethonium inhibit ACh stimulation of the pain nerves — see Figs. 27 and 28, where $10^{-5}$ g/ml. of tubocurarine or decamethonium respectively — have antagonised $10^{-4}$ g/ml of ACh. Hexamethonium on the other hand, antagonises the action of acetylcholine only if applied in high concentration ($10^{-3}$ g/ml.) but its activity
is low as compared with that of d-tubocurarine and decamethonium.

Though atropine antagonises ACh on the blister area it does so only when its concentration is greater (say fourfold) than that of acetylcholine. Atropine is, thus, inactive at $10^{-5}$ g/ml. concentration and becomes active at $10^{-4}$ g/ml. It is well-known that pharmacological actions lose their specificity at high concentrations and atropine is no exception since in high concentration it antagonises both 'muscarinic' and 'nicotinic' actions of ACh. Thus, whilst 'it is probable that the action of ACh at the ending of each individual nerve in each species of animal should be regarded as a distinct problem in itself' (Barlow, 1955) it would seem that the ineffectiveness of methacholine and pilocarpine in stimulating pain, together with the much lower activities of atropine and hexamethonium as antagonists, as compared with the activities of d-tubocurarine or decamethonium indicate that the pain nerve endings closely resemble motor end-plates in their properties.

The following evidence suggests that these properties may be regarded as normal and not induced by a specialised state:

1. I have shown (Part I, Methods) that the
cantharidin blister area responds to acetylcholine and other chemical stimuli as does the base of a blister raised by heat.

2. Brown and Gray, working on the chemical stimulation of skin sensory nerve preparations (of dog and cat) where the nerve endings are normal and undisturbed, found results which correlate with those on the cantharidin blister area:

a. a stimulant action by $10^{-4}g/ml. \text{ACH}$,  
b. a stimulant action by carbachol when its concentration = $\text{ACH} \times 10$. This is in agreement with blister area findings,  
c. a stimulant action by nicotine in $10^{-4}g/ml.$ concentration and  
d. lack of stimulation by methacholine.  
(Brown & Gray, 1947).

iii. The Rôle of Acetylcholine in the Production of Pain.

The following factors are worth considering when assessing the rôle of ACh in the production of pain in the skin:

1. Acetylcholine has been shown to be amply capable of stimulating a wide variety of nerve structures as well as to produce human pain, e.g.

a. spinal cord (Bubring & Burn, 1941),  
b. ganglia (Bronk, 1939; Feldberg & Vartiainen, 1935; Feldberg & Hebb, 1948),
c. motor nerves (Masland & Wighton, 1940)
and, perhaps most important of all
d. sensory nerve origins (Brown and Gray,
1947; Douglas and Gray, 1952). The
effective concentrations of the drug
required for these actions are of the
same order, $5 \times 10^{-6}$ to $5 \times 10^{-5}$g/ml.

2. Cholinesterase, true and specific, has
been found there (Thompson & Whittaker,
1944). Its content increases after
irradiation of the skin, peak
concentrations being reached in the first
twenty-four hours after burning (Baglioni
& Piemonte, 1947) - the period when pain
and hyperalgesia of the skin may be
expected to be most severe.

3. Choline is present, mostly bound, in
human skin (post-mortem, abdominal) in
high concentration about 1.4 mg/g. wet
weight). The epidermis contains about
six times as much as the dermis and
twenty times as much as the stratum
corneum. In certain painful
conditions (exfoliative dermatitis and
inflammatory sunburn reaction) there
is transference of choline towards
the stratum corneum and liberation

\[ \textit{theeze, part III}. \]
of free choline in the epidermis where its concentration rises manifold (Snider, Gottschlak and Rothman, 1949).

4. **Choline-acetylase** has been found in other non-nervous tissue (Chang & Gaddum, 1933) but it has not yet been found in skin.

5. It has been found possible to lower the skin pain-temperature threshold by intradermal introduction of acetylcholine or prostigmine and to raise it by means of local administration of atropine and it has therefore been suggested that (1) "Changes in the concentration of the active acetylcholine normally liberated in the skin may influence the sensitivity to pain", and (2) "that the activity of the pain receptors is" possibly "influenced by changes in the acetylcholine content of the skin" (Skouby, 1951).

In the light of present knowledge it would seem probable that acetylcholine may influence the skin sensitivity to painful stimuli though it would seem to play a lesser rôle in the direct stimulation of pain in the skin than do certain tissue-injury products to be described (thesis, Part III).
Solutions of KCl induce pain in the blister area in concentrations down to about $10^{-3}$ g/ml. (1/10 isotonic). The pain, unlike the immediate brief type shown by a wide range of concentrations of ACh, usually takes two or three seconds to occur, mounts somewhat slowly, slowly declines and altogether lasts two or three times as long as ACh-induced pain of similar intensity.

In Fig. 32 the rates of onset and decay of ACh and KCl induced pain are compared. The (a) tracing of that figure, subject J.W.M. shows the differing time-courses of pains of equal intensities caused by the two agents.

In the (b) tracing, (CAK.) two pain-intensity pictures to two concentrations of KCl are compared with the response to one concentration of ACh, the responses to KCl being one greater and one less than that of the ACh. This record is made on a fast-moving drum to accentuate the differences in time-course.

The long drawn-out responses to KCl can be clearly seen occurring both to the concentration of KCl producing greater and that causing lesser pain than the ACh. Similarly for the rate of rise of the KCl pain, even in the concentration producing immediate onset of pain which reaches even greater intensity than the ACh pain, the rate of rise is slower.

As with ACh administration, the responses are graded
in proportion to the concentration of KCl applied. 78. Following the administration of KCl the area has a refractory period to KCl (and to ACh). The refractory period to KCl is of similar duration to that found for ACh in the study of the Method and was illustrated in that study (p. 40). Following administration of $7.7 \times 10^{-3}$ g/ml. (2/3 isotonic) KCl, recovery was observed to be incomplete at 5 minutes, but to be complete at 10 minutes after washing off. The action of this substance is probably on the nerve fibres and this is illustrated in Figures 16 and 17 where, in Fig. 17, the failure of ACh to cause pain during the refractory period immediately following KCl administration, and the success of KCl Fig. 16, in causing high-intensity pain during the refractory period of the area to ACh are shown.

3. SYMPATHOMIMETIC AMINES.

Adrenaline and Nor-adrenaline.

1-Adrenaline d-tartrate and 1-nor adrenaline do not produce pain at $10^{-4}$ g/ml. concentration, when applied to subjects responding to $2 \times 10^{-5}$ g/ml. concentration of ACh. (Fig. 33 (a) and (b)).

In $10^{-3}$ g/ml. concentration both adrenaline and nor adrenaline did cause pain. This was due neither to pH nor to the tartrate content of solutions used.

Ephedrine

Ephedrine HCl also, at $10^{-4}$ g/ml., did not cause pain
Table 3. HISTAMINE.

<table>
<thead>
<tr>
<th>HISTAMINE CONCENTRATION estimated as base g/ml.</th>
<th>PAIN</th>
<th>EFFECTS</th>
<th>ITCH</th>
<th>SUBJECTS</th>
<th>ADMINISTRATION</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>(½ + on scale)</td>
<td>Doubt-ful</td>
<td>Abs-ent</td>
<td>(0, on scale)</td>
<td>Number of:-</td>
</tr>
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<td>4</td>
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<tr>
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<td>4</td>
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</table>
when tested on an area responding to $ACh$ $10^{-4}$ g/ml. and 5-HT $10^{-6}$ g/ml. with pain of 3 and 1 unit respectively. (Fig. 33c)

4. **HISTAMINE.**

**A. Applied Histamine.**

Histamine can produce pain or itching depending on the concentration. The following table, Table 3, summarises the results of 55 administrations of histamine to 9 subjects.

**Pain.**

It can be seen that out of 14 administrations of histamine of concentration $10^{-8}$ to $10^{-6}$ g/ml. none produced definite pain (i.e. pain of $\frac{1}{2}$ unit or over - see p. 37, Methods) though 7 of these administrations were at the high level of $10^{-6}$ g/ml. $10^{-5}$ g/ml. histamine is the lowest concentration at which definite pain occurred (i.e. pain of $\frac{1}{2}$ unit or over) but out of 14 administrations at this concentration less than half, 6, were positive effects, and an equal number experienced no pain at all.

With a tenfold higher concentration of histamine - $10^{-4}$ g/ml. there were still as many completely negative results as positive ones. At $10^{-3}$ g/ml. there were more painful reactions to histamine than non-painful ones though, even with this high concentration, 5 of the 16 administrations
did not cause pain.

Itching.

In contradistinction to its poor pain-producing qualities it can be seen, in the same table, that histamine, administered in this way, in a concentration of $10^{-5}$ g/ml. upwards, more usually produced itch than not, and at $10^{-3}$ g/ml. almost all applications produced definite itch.

The threshold concentration for itch production is apparently about $10^{-7}$ g/ml. and for pain production it is about $10^{-5}$ g/ml. The pain, when it occurs, is early in onset and does not compare in intensity and duration with that induced by similar or lower concentrations of ACh or 5-HT or of active agent from blister fluid. (see below, p. 100). (Fig. 34)

In a series of applications of histamine ($10^{-5} - 10^{-3}$ g/ml.) to five subjects ten out of 18 administrations produced pain, and in 9 out of the 10 the pain responses occurred with less than 15" delay, two of these being immediate responses. Only 1 of the 10 pain responses occurred at over 1 min. In contrast 11 of the 13 itch responses occurred at one minute or over and the remaining two occurred at 30".

I performed two experiments to see if I could demonstrate a potentiating action by histamine of pain of threshold intensity induced by
acetylcholine. Having found the concentration of acetylcholine which gave a constant and threshold effect, histamine and acetylcholine were then mixed in suitable proportion to give the required final concentrations of each in the other and applied thus together. Fig. 35 (a) (R.M.L.D.) shows that the threshold pain-response to $10^{-5}$g/ml. ACh was not increased when $10^{-4}$g/ml. histamine (dihydrochloride – estimated as base) was administered with it. When the acetylcholine was administered with $10^{-3}$g/ml. histamine marked pain was caused but this was shown to be due to the histamine content itself and not to the mixture – Fig. 35 (b). The failure to potentiate the ACh action cannot be attributed to a low slope of dose-response curve for the area since increase in ACh 'dose' from $10^{-5}$g/ml. to $2.5 \times 10^{-5}$g/ml. increased the pain produced from less than a unit intensity to two units intensity.

Thus, in addition to being non-pain-producing in low concentrations histamine ($10^{-8}$ to $10^{-5}$g/ml.) is also not a potentiator of ACh even when administered in high concentrations (e.g. $10^{-4}$g/ml.)

B. Histamine Liberation.


The powerful histamine - liberating compound, 48/80 (a condensation product of p-methoxy
phenoxy ethyl methyamine and formaldehyde) causes delayed fluctuating and prolonged pain and also itch when applied to the blister base. Fig. 36. The pain begins at something over half a minute after application of the solution and itch follows about a minute later. Marked flare and wheal were seen.

2. Tryptamine and 5-hydroxytryptamine.

Though tryptamine and 5-hydroxytryptamine are histamine-liberators (Feldberg and Smith 1953b), and pain-producers (see below, p. 85) no itch, wheal or flare has followed their application. It may well be that the reason for this is that their histamine-liberating potency is only of the order of one-hundredth of that of compound 48/80 (Feldberg and Smith, 1953a). The pain-producing potency of tryptamine and 5-hydroxytryptamine is, however, at least one hundred times greater than that of compound 48/80 and so, correspondingly lower concentrations of the tryptamine are administered to produce pain. Thus, the concentrations of tryptamine and 5-hydroxytryptamine effective to produce marked pain when applied to the blister area, liberate less than one ten-thousandth the amount of histamine produced by only moderately-effective pain-producing
concentrations of compound 48/80. The pain produced by tryptamine and 5-hydroxytryptamine may therefore be regarded as not being dependent on the histamine liberated by them.

Discussion

On the blister base histamine often produces pain concentrations above $10^{-5}\text{g/ml}$. It regularly produces itching in concentrations of $10^{-5}\text{g/ml}$ and above, and sometimes in concentrations down to $10^{-7}\text{g/ml}$. Pain when it occurs, develops early, within 1 to 15 sec., whereas itching usually develops about 1 minute after application. These findings do not agree with those of Rosenthal and Sonnenschein (1948) who reported that intradermal injection of histamine produced pain in lower concentrations than those required for itching, and claimed that histamine could evoke pain in a concentration of $10^{-18}\text{g/ml}$. Broadbent (1953) has suggested that histamine only produces pain when introduced into the dermis and that when applied to the epidermis (as by pricking through a drop of solution on the skin) it produces itching. My findings with histamine could be explained in different ways:

1. The cantharidin blister exposes itch endings in the deeper epidermis, but does not sufficiently expose the pain endings in the
dermis, to the actions of histamine.

2. The pain nerve endings exposed by the blister are less sensitive to histamine than the nerve endings which subserve itching.

3. Some blister bases may contain no itch nerve endings at all, since complete removal of the epidermis abolishes the sensation of itch (Rothman, 1943). These bases may thus respond only to concentrations of histamine high enough to cause pain. Itching could result from spread of histamine from the exposed base into the epidermis of the surrounding intact skin.

The possibility that histamine would potentiate the pain-producing action of ACh on the blister base was studied in view of the finding of Emmelin and Feldberg (1947a) that such potentiation occurred when these two substances were applied by pricking into the epidermis. Emmelin and Feldberg's results might be due to the enhancement by histamine of penetration of ACh to the pain nerve endings in the dermis.

The actions of compound 48/80 might be due to liberation of high local concentrations of histamine which could produce both pain and itching. However, 48/80 might also liberate other pain-producing substances, such as 5-hydroxytryptamine (Feldberg and Smith, 1953a) or it
<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2/&lt;/sub&gt;</th>
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<td>Tryptophane. β indolylamine</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;.CH.CO&lt;sub&gt;2&lt;/sub&gt;.NH&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>H</td>
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<tr>
<td>Tryptamine. β indolyl ethyl</td>
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<td>H</td>
<td>H</td>
<td>H</td>
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<tr>
<td>4-hydroxytryptamine,</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;.CH&lt;sub&gt;2&lt;/sub&gt;.NH&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>H</td>
<td>H</td>
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<td>4-HT.</td>
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<td>5-hydroxytryptamine,</td>
<td>H</td>
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<td>6-hydroxytryptamine,</td>
<td>H</td>
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<td>6-HT.</td>
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<tr>
<td>NN-dimethyltryptamine.</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;.CH&lt;sub&gt;2&lt;/sub&gt;.N(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>αα &quot; &quot; &quot; &quot; αα &quot; &quot; &quot; &quot;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;.C(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;.NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
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<tr>
<td>ββ &quot; &quot; &quot; &quot; ββ &quot; &quot; &quot; &quot;</td>
<td>C(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;.CH&lt;sub&gt;2&lt;/sub&gt;.NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Bufotenin. 5-hydroxy NN</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;.CH&lt;sub&gt;2&lt;/sub&gt;.N(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
<td>OH</td>
<td>H</td>
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<tr>
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<td>Gramine. β indolyl methyl</td>
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<td>H</td>
<td>H</td>
</tr>
<tr>
<td>dimethyl amine.</td>
<td></td>
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may be merely pain-producing in itself.

Pain production by 5-hydroxytryptamine is not
due to histamine liberation.

5. INDOLEALKYLAMINES

A. A Study of the Actions of Various Amines.

Figure 37 (R.M.I.D.) shows that tryptophane is
not pain producing and has a potency of one-
hundredth or less that of the corresponding amine,
tryptamine, which is the most potent agent of
known composition mentioned so far in the thesis.
The figure shows that there was no pain response
to $10^{-3}$ g/ml. tryptophane in an area responding to
$5 \times 10^{-5}$ g/ml. ACh and $10^{-5}$ g/ml. tryptamine HCl.
Tryptamine is in fact a very potent pain-producing
agent and Figure 38 (R.M.I.D.) shows pronounced,
delayed and long-lasting pain induced by $10^{-6}$ g/ml.
tryptamine HCl on an area of threshold sensitivity
to acetylcholine around $10^{-5}$ to $2.5 \times 10^{-5}$ g/ml.

Introduction of an hydroxyl group in the 5 position
of the indole ring results in an even more potent
pain-producing agent, namely,

$$5\text{-hydroxytryptamine} \quad \begin{array}{c}
\text{HO} \\
\begin{array}{c}
\text{CH}_2\text{CH}_2\text{NH}_2 \\
\text{NH}
\end{array}
\end{array}$$

5-HT

Serotonin

In Fig. 39 (a) (J.W.M.) is shown a record of
severe pain induced by $10^{-7}$ g/ml. 5-HT creatinine
sulphate. This substance can cause pain even in
concentrations as low as $10^{-8}$ g/ml. (equivalent to
$5 \times 10^{-9}$ g/ml. 5-HT base) as shown in (b) of the same figure, Fig. 39. The increased potency of the 5-OH compound over the unsubstituted tryptamine is apparent even in the face of the depression of the area which these agents cause (see Fig. 10) and the ratio of potencies of the compounds is shown, in Fig. 39 (b), to be at least twenty to one in favour of the 5-hydroxy compound.

Just as tryptophane is less active than the corresponding unsubstituted ethyl amino side-chain indole, tryptamine, so are the $\alpha\alpha$ dimethyl amino, and the $\beta\beta$ dimethyl amino substituted tryptamines less active than tryptamine itself, indicating the desirability of an unsubstituted side-chain for high pain-producing potency. The effect of the $\alpha\alpha$ dimethyl substitution is shown in Fig. 40 where $10^{-5}$M tryptamine is more active than $10^{-4}$M of the substituted compound and less active than $10^{-3}$M, making the unsubstituted tryptamine more than ten but less than one hundred times as active as the dimethyl tryptamine.

With the $\beta\beta$ dimethyl compound the ratio of potencies was somewhat over one hundred to one in favour of the unsubstituted tryptamine.

The preference for free side-chain over dimethyl substitution is shown up once again, this time with respect to the amino group, when the hydroxy
compound, **bufotenin**, 5-OH.NN dimethyl tryptamine, from toad skin secretion is compared with 5-hydroxytryptamine, from beef serum (Rapport Green, and Page, 1948) and found to be considerably less active - Fig. 41 (a) (D.A.). Though in Fig. 41(a) bufotenin is apparently a feeble pain-producing agent it is in fact of fairly potent since $10^{-6}\frac{g}{ml}$ is frequently capable of producing pain. In Fig. 42, (Read) $10^{-6}\frac{g}{ml}$ bufotenin is shown to cause pain on an area where $5 \times 10^{-5}\frac{g}{ml}$ ACh produced pain of similar intensity though shorter duration.

Again, with regard to the potentiating effect of introducing an OH group in to the indole ring, just as 5-hydroxytryptamine is more potent than tryptamine so 5-hydroxy NN dimethyl tryptamine is more active than NN dimethyl tryptamine. This is shown in Fig. 41 (b) where the effects of equal concentrations were compared. The compound lacking the hydroxyl group produced no pain, whilst bufotenin itself caused severe or moderate pain.

The 5 position is probably optimal for the placing of the OH group in the indole ring, since both 4 and 6 hydroxytryptamines are definitely less active than 5-HT, 6 being the least active of the three. Figure 43(J.B.J.) shows that 5-HT is
more potent than 4-HT (by comparison of responses to $5 \times 10^{-6}$ M). It is, however, not as much as ten times more potent since $5 \times 10^{-5}$ M 4-HT more than equals $5 \times 10^{-6}$ 5HT, even though the former comes later in the tracing and would be expected to have been antagonised to some extent by the preceding 5-HT administration 4-and 5-hydroxytryptamines are thus of similar high pain-producing capacity, 4 being somewhat less potent than 5. 6-HT, however, has less than one tenth but more than one hundredth of the potency of 5-HT. This can be seen in Fig. 44 (J.B.J.) when the responses to $5 \times 10^{-6}$ M and $5 \times 10^{-5}$ M 6 HT are compared with those to $5 \times 10^{-7}$ 5 HT.

Thus it would seem that both the side-chain (preferably ethyl amino unsubstituted) and the indole group (preferably OH substituted) make an important contribution to high pain-producing potency.

On the basis of these observations, another indole compound, gramine, lacking the highly active ethyl amino side chain, lacking any OH in the indole ring, and having a dimethyl substitution in the amino group (cf. NN dimethyl tryptamine) would be expected to have a feeble pain-producing action, much less than that of 5-hydroxytryptamine. The prediction is indeed correct as shown in Fig. 45 where gramine $10^{-5}$ g/ml. did not cause pain though
$10^{-6}$ g/ml. 5 HT did. Applied as bathing fluid, gramine, $10^{-5}$ g/ml. did not block the pain-response to subsequent application of 5-HT though 5-HT applied as bathing fluid effectively blocked against itself. Thus gramine neither produces "tryptamine-like" pain nor blocks it.

Of the indole compounds tested 5-hydroxytryptamine has the optimum structure for pain-production. It is the most potent pain-producing agent of known composition mentioned in the thesis.

Tryptamines and Serum.

The marked similarity between the pain caused by the application of active tryptamines and that caused by application of a subject's own serum to his blister area is seen when Fig. 38 tryptamine, is compared with Fig. 54 serum, or when Fig. 46 (a and b), 5-HT and serum, respectively are compared. The pain due to tryptamine and to serum are similar in time-course and in character also, and are clearly different from pain caused by ACh and KCl. Most important is the similar selective progressive depression of the blister area which these agents produce - see Fig. 10 where progressive depression of the blister area responses to serum and to 5-HT occur. The depression is of a degree such that over ten times the concentration of 5-HT would have to be administered to produce the original intensity of
pain occurring after the same latent period. Meanwhile the response to a single dose of ACh had changed by only 40 - 20% by the end of the experimental session. This suggests that a tryptamine may be the pain-producing substance in human serum - perhaps the 5-OH compound serotonin, as in beef serum.

B. Antagonism of tryptamine-induced Pain-responses. The blockade of one tryptamine by itself is fairly easy to study since all one has to measure is the falling-off in pain response with repeated applications of that substance, and to test the specificity of the block by observing the effect of the test-substance on pain responses to other agents, as was done in the experiment recorded in Fig. 10.

However, with such self-blocking materials, it is difficult to study the antagonistic effects of other chemicals on them since, without an original response due to the tryptamine, there is no measure of the percentage inhibition of response caused; alternatively, after an initial measure of protagonist response any reduction in tryptamine response following administration of antagonist is then due to the sum of inhibitions caused by pro and antagonists. I have studied this problem only briefly, using the first method - that of first applying the supposed antagonist and observin
the order of intensity of the response induced by the protagonist whether it be good, poor or absent. Using the following antagonists as described in Methods, p. 57:

1. 5 HT - $10^{-6}$ g/ml.
2. Bufotenin $10^{-6}$, $10^{-5}$ g/ml.
   
3. 5 Amino
   
   3 Ethyl
   
   2 Methyl
   
   Indole $10^{-6}$ g/ml.

4. D.H.E. $10^{-5}$ g/ml.

I found the pain responses to subsequently administered 5-HT $10^{-6}$ g/ml. to be absent or very poor.
Gramine $10^{-5}$ g/ml. was an ineffective antagonist to 5-HT. In addition, tryptamine and bufotenin each produce a self-block.

C. Conclusion.

1. Tryptamines include the most active pain-producers of known composition and can be effective in a concentration of 1 ug/ml. or less.

2. For high pain-producing activity the optimal chemical structure is a 4 or 5-OH indole ring with an unsubstituted 3-ethyl amino-side chain.

3. Of the tryptamines tested the 5-hydroxy compound, serotonin, is the most potent. Significant responses have been recorded to $5 \times 10^{-9}$ g/ml. 5-HT and the compound has therefore the highest pain producing activity.
among substances of known composition which I have tested.
The indole compounds described in this section may be divided into three main groups according to their power to produce pain when applied to the blister base:

1. 5-hydroxytryptamine — serotonin
   4-hydroxytryptamine
   Tryptamine
   5-hydroxy NN dimethyl tryptamine — all active
   bufotenin 10^6 g/ml.

2. 6-hydroxy tryptamine
   αα-dimethyl tryptamine
   γγ-dimethyl tryptamine

3. NN dimethyl tryptamine
   indolyl methyl dimethyl amine — NOT active at
   gramine
   indolylα amino propionic acid—
   tryptophane
   10^-5 g/ml.

4. Tryptamines are particularly liable to produce depression of the blister area responses to themselves and to other compounds of the group. The depression is selective as the pain-response to acetylcholine is not similarly antagonised.

5. The pain-response to tryptamines resembles that due to serum in two important respects:
   i. time-course and character.
ii. the similar selective depression of the blister-area responses which follows, and suggests that the pain-producing agent of serum could be a tryptamine. (The pain-response to blister fluid, however, though resembling that due to tryptamine and serum in time-course and character differs from it in the absence of marked depression of the area following its administration).

6. ADENOSINE TRIPHOSPHATE.

Adenosine triphosphosphate was used as the disodium salt and applied to blister areas in increasing concentration. The figure, Fig. 47 shows that on an area, sensitive to $10^{-5}$ g/ml ACh and very sensitive to $10^{-6}$ g/ml. 5-HT, $10^{-5}$ g/ml. ATP produced no pain and even $10^{-4}$ g/ml. caused only slight pain. ATP cannot therefore be regarded as a potent pain-producing agent.

7. HYDROCHLORIC, LACTIC AND CITRIC ACIDS.

See Part I - Methods, 7, p.

8. POSTERIOR ROOT EXTRACT.

(Hellauer and Umrath, 1947
Holton and Holton, 1952).

Figure 48 (a) and (b), J.W.M. shows that a sufficiently high concentration of the dried acetone extract of posterior-root nerves of horses, re-constituted in saline, e.g. $5 \times 10^{-3}$ g/ml. will produce
moderate to severe pain of almost immediate onset. $10^{-4} g/ml.$ of the extract produced no pain on an area responding to $5 \times 10^{-5} g/ml.$ ACh and $10^{-7} g/ml.$ 5-HT. The posterior-root powder used corresponded to five times its weight of parent dorsal root. If the extraction rate of the active principle were comparable with that for ACh from nervous tissue (say $10^{-5} g/g$ wet weight) then effective concentrations administered would be well below $1 g/ml.$ in solution which would represent a potent pain-producing substance. The nature of the active principle is not yet known though its investigators have already eliminated ACh, histamine, adenosine, substance P (Gaddum and Schild, 1934) bradykinin (Rocha e Silva Beraldo and Rosenfeld, 1948) kallikrein (Kraut, Frey, and Werle, 1933) and necrosin (Menkin, 1943) as being the active substance (Holton and Holton, 1952) and have later observed a relationship between the active substance and ATP. The relationship of the posterior-root extract to sensory nerve stimulation is not clear and, since similar amounts to those found in dorsal roots have been found in ventral roots its specific function is questionable (Holton and Holton, 1952). Its significance in the production of pain in man is likewise questionable.

9. CERTAIN POLYPEPTIDES.

Various polypeptide preparations applied to the blister-base caused pain. Actions of:—
Hypertensin (Angiotonin) - Corcoran and Bumpus - a lyophilised sample prepared by the action of hog renin on horse globulin - 10 units /mg,
Bradykinin - Rocha e Silva - bovine origin, purified to P.P.M. stage,
Substance P - Pernow; 10 units/ mg,
Vasopressin - pitressin - Roche, and
Leukotaxine - Menkin,
are illustrated (Figures 49 (a) and (b) and 50 (a) and (b)). The pain when it occurs takes time to appear, builds up slowly and lasts for a long time (several minutes). The records show pain experienced during two minutes only, in order not to overtax the area, after which recording stopped and the active solutions were replaced by bathing fluid. Unlike the tryptamines these substances have little blocking action against themselves or again one-another.
Of all the substances tested, the polypeptides (with their delayed, slow-mounting, long-maintained pain, not followed by acute refractoriness of the area, and without liberating histamine) most closely resemble blister fluid in its pain-producing actions when it is applied to the blister base.

10. CONCLUSION.
1. Many types of substance are capable of inducing pain but only a few can be relied on to cause pain when applied to the blister area in a concentration of $10^{-4}g/ml$. 
e.g. acetylcholine
carbachol
nicotine
compound 48/80
certain tryptamines
adenosine triphosphate
(histamine)
Fewer will cause pain at $10^{-5}$ g/ml.

e.g. acetylcholine
certain tryptamines.

The following substances were inactive at the concentrations stated.

- histamine at $10^{-5}$ g/ml.
- adrenaline
  - nor-adrenaline at $5 \times 10^{-4}$ g/ml.
- methacholine
- pilocarpine at $10^{-3}$ g/ml.
- tryptophane

Of the substances mentioned, only the tryptamines (notably 5-hydroxytryptamine) are sufficiently powerful to induce pain when they are present in concentrations of $10^{-6}$ g/ml. and below.

Solutions of pH3 and below will be expected to produce pain on application.

Certain Polypeptide Preparations eg.

Vasopressin (Pitressin)
Bradykinin
Angiotonin
Leukotaxine
produce pain when they are applied to the blister area in concentrations of $10^{-4}$ to $10^{-5}$ g/ml. (unpurified powders). Since the purity of these preparations is low and the molecular weights of the active principles are high, the activity of these substances as compared with those of the various low-weight crystalloids must be very high indeed, being probably hundreds of times more active than 5-hydroxytryptamine and acetylcholine and thousands of times more active than adrenaline, nor-adrenaline and histamine.

2. a. Though a substance will produce marked pain a close chemical relative may be inactive e.g. i. acetylcholine and methacholine. ii. 5-hydroxytryptamine and NN dimethyl tryptamine.

b. The cantharidin blister area technique for the testing of pain-producing substances may be used to study the relation of structure to pain-producing action in groups of compounds. This has been demonstrated in this section, Part II, for compounds related to acetylcholine and 5-hydroxytryptamine.

3. The pain caused by application of tryptamines or polypeptides to the blister area is delayed in onset, slow-mounting and long maintained. The pains produced by these substances are not distinguishable from one another (though they are,
of course, easily distinguished from the sharp immediate pain induced by acetylcholine, hypo-
or hyper-tonicity or acidity) nor are they distinguishable from pain induced by serum, blister fluid or pleural effusion. Repeated applications of the tryptamines or serum produce marked refractoriness of the area. This does not happen with polypeptides or blister fluids. It is suggested that 5-hydroxytryptamine may be the agent responsible for the pain induced by serum and that of blister fluids may be due to some other agent capable of producing delayed prolonged pain without inducing marked refractoriness of the area, e.g. a bradykinin-like substance.
PART III.
PART III - PAIN PRODUCTION BY

a. HUMAN INFLAMMATORY EXUDATES.

b. 'DAMAGED' HUMAN BLOOD.

c. SKIN EXTRACTS.

1. DEMONSTRATION.

2. SUPPLEMENTARY BIOLOGICAL TECHNIQUES USED IN THE STUDY OF THESE PAIN EXCITANTS.

3. EXPERIMENTS CARRIED OUT ON THE CANTHARIDIN BLISTER AREA AND ISOLATED RAT UTERUS USING THE FLUIDS OF PATHOLOGICAL ORIGIN AND/OR CHEMICAL DERIVATIVES FROM THEM, IN AN ATTEMPT TO ELUCIDATE THEIR CHEMICAL NATURE.

4. SUMMARY.

5. DEMONSTRATION OF, AND COMMENTS ON PAIN PRODUCTION DURING AND FOLLOWING CUTANEOUS HEAT BURN OF THE FOREARM.
PART III - PAIN PRODUCTION BY

a. HUMAN INFLAMMATORY EXUDATES
b. 'DAMAGED' HUMAN BLOOD
c. SKIN EXTRACTS.

1. DEMONSTRATION: Pain-production, by

a. Human Inflammatory Exudates

When various pathological fluids were collected from man in to a glass syringe and applied to a blister area a few minutes later the subject experienced well-marked pain.

The pain-producing actions of human inflammatory exudates are shown in the following Figures:

- Cantharidin-blotch fluid  - Fig. 18A
- Burn-blotch fluid  - " 18B
- Insect-bite-blotch fluid  - " 51A
- Rheumatoid arthritic joint fluid  - " 51B
- Pleural effusion  - " 51C

The characteristics of the pain are not distinguishable from those of serum, the pain being delayed (delay up to about 45"), slow-mounting and long maintained. (c.f. Fig. 54.)

Sometimes an administration of one of these pathological fluids failed to give pain. Inspection of the data soon showed that the active principle was unstable since the fluids which had stood for an hour or two at room temperature (20°) did not produce pain. Figure 52 shows the effects
of the same cantharidin blister fluid applied fresh, (a), and after keeping for three hours at room temperature, (b). Storage at 0° delayed the decay, and at -20° maintained the activity. If an aliquot were removed from such stores at -20° and left at room temperature for an hour or more whilst the remaining solution was left in the frozen condition, the decay of activity could be demonstrated in one experimental run. It was in that manner that Figs. 51A and 53 were made. Figure 53 shows the inactivity of burn-blister fluid one and a half hours after removal from the storer, whilst another aliquot of the same fluid, applied to the blister-area five minutes after unfreezing, caused very severe pain of typical delayed (25" delay) slow-mounting type. Similarly, Fig. 51A, administration 2, shows the effect of insect-bite blister fluid kept at room temperature for three and a half hours whereas the cold-stored material was still active in the typical manner.

b. 'Damaged' Human Blood.

i. Fresh Blood straight from the Body.

When blood is carefully collected into a cold syringe, and immediately applied to the exposed blister-base it does not cause pain. (See also p. 117 et seq.)
ii. Serum and Saline Extract of Broken Platelets.

Serum is markedly pain-producing. Its actions are shown in Figures 46 and 54. Plasma (heparinised or citrated), of similar age (2 - 3 hr. old) is inactive; Fig. 55 shows the pain-producing actions of plasma and serum at two hours. The delayed slow-mounting pain is very similar to that produced by the tryptamines and by the pathological effusions and certain polypeptide preparations. However, the activity of serum differs from that of the exudates and polypeptides, in that refectoriness of the area develops on repeated application. (see Methods, p. 40) Furthermore, unlike the activity of the exudates, that of serum is stable during several hours at room temperature, 20°C, (see decay of the activity of blister fluids, p. 113). The activity is dialysable (Fig. 56). The dialysate is stable and can be maintained at pH 1.5 or 9.5 for 24 hr.

Addition of an anticoagulant to serum does not reduce its activity. At that time I concluded that the negativity of the plasmas tested was most likely due to inhibition of platelet breakdown in them, by the anticoagulants, and that the activity of serum might be due to liberation of a pain-producing agent from
damaged platelets. The effect of an extract of broken platelets on the blister area was then investigated.

Platelet extracts were kindly prepared for me by Dr. J.W. Stewart, of the Bland Sutton Institute of Pathology, in the following manner:

1. The blood was collected and equally divided into two tubes containing sodium citrate to give a final concentration of $4.7 \times 10^{-3}$ g/ml.

2. One was well centrifuged (3000 rev/min for 25 min) and the supernatant carefully aspirated and transferred to a haematocrit tube A.

3. The other was (i) allowed to stand in the warm (for 25 min) and (ii) the supernatant transferred to a haematocrit tube which was (iii) spun lightly (200 rev/min for 5 min). The plasma was transferred to a fresh haematocrit tube – tube B.

4. Tubes A & B were then both well spun (3000 rev/min for 20 min) and almost all the plasma (98%) of both tubes was discarded.

5. CaCl$_2$ solution (0.01 molar was added to both tubes in equal volume, the 'deposits' stirred up, and 0.9% NaCl added to make the volume up to 100%.

6. After standing (for 15 min) and spinning at low speed (200 rev/min for 3 min) the supernatants which were quite clear were transferred to clean, stoppered bottles:
A = Control
B = Test extract of platelet rich, leucocyte poor (or absent), red cell free plasma.

Figure 57 shows that when such a platelet extract was applied to the blister-area pain was caused (response 3) whilst the control solution, made from platelet-poor plasma did not cause pain (response 1). The pain response was of the delayed type, slow mounting and long lasting (as for serum). The potency of the solution tested was approximately equal to $5 \times 10^{-8}$ g 5-HT base/ml. blood.

5-hydroxytryptamine has been identified in the platelets of various animal species by both pharmacological and chemical techniques (bovine - Rand and Reid, 1951; Zucker, 1954; rabbit and sheep - Bracco and Curti, 1953) and in human platelets by pharmacological techniques, (Humphrey and Jaques, 1954 - Borelli and Zucker, 1954; Hardisty and Stacey, 1955) and the values of the human platelet agent measured against 5-hydroxytryptamine on the several biological preparations have been in the range $5 \times 10^{-8}$ to $6 \times 10^{-7}$ g 5-HT base per ml. blood.

In view of Hardisty and Stacey's findings (1955) that there is 'considerable variation in the concentration of 5-HT in the blood of
different normal individuals', and that 'this cannot be explained solely by differences in the number of platelets present, since the amount of 5-HT per platelet shows an equally large spread', the activity of the platelet extract, tested on the pain area, in terms of 5-hydroxytryptamine, seems to be of the same order as the yield of 5-HT observed by others for human platelets.

Moreover, it has been found that in normal human subjects:

1. blood prior to coagulation 'contains almost no 5-HT',
2. on coagulation there is considerable activity present (in terms of 5-HT, $2.5 \times 10^{-7}$ gm base/ml) as observed on the ox carotid,
3. serum or blood artificially deprived of platelets prior to coagulation is almost inert (Bigelow, 1953).

Thus, 1) potential activity (for contraction of carotid ring, and pain production) appears on the clotting of human blood.

2) the potency of the active agent, in terms of 5-HT base, as measured on both preparations, is of the same order (about $2.5 \times 10^{-7}$ gm/ml.) and

3) The activity of the serum (on carotid ring preparation) is proportional to its
platelet content, whilst
4) an extract of platelets applied to
the blister area is pain producing,
5) 5-hydroxytryptamine, in like
concentration, is also similarly pain
producing,
and so it seems reasonable to believe that
pain caused by human blood when it clots is
due to liberation of 5-hydroxytryptamine found
in human, and other, platelets and liberated
from them in clotting.

iii. Human Plasma.
In early experiments it was found that fresh
blood straight from the body, and plasma two
hours old, (Fig. 55 ) did not produce pain.
This seemed to contrast appropriately with the
pain-producing action of serum, which contains
5-HT released from platelets during clotting.
It was therefore very surprising to find that
in later experiments application of plasma
to a blister area produced marked pain.
Further studies showed that when plasma was
tested between 5 and about 60 minutes after
removal from the body, it produced pain
similar in type to that produced by inflammatory
exudates (see p. 100 ). The pain was
produced by both heparinised and citrated
plasmas of suitable age (Fig. 58 (a) and (b)
respectively) and was not caused directly by either anticoagulant. Thus plasma, with anticoagulant contained an unstable pain producing substance, though addition of anticoagulant to serum did not lead to decay or increase of serum activity. Thus, two powerfully pain-producing substances could arise in shed blood - one, apparently 5-HT from the platelets, which is stable; the other unstable and, at this stage, of source unknown. The unstable pain-producing agent in human plasma and inflammatory exudates will be referred to as P.P.S. (Pain Producing Substance).

iv. Heated Human Plasma.

Fig. 59 shows that when human plasma in a polythene container was heated (in a waterbath) at 70°C and applied to a blister area, it caused pain. The pain was delayed and prolonged at peak. Recording during one minute is shown. The same plasma kept similarly at 37°C and tested at intervals from 2 to 20 minutes warmth exposure caused no pain.

c. Skin Extracts.

Extracts of (i) rat and (ii) human skin were made by cutting up the cleaned skin in Ringer Locke solution with scissors. The process took from 10 - 30 min. according to the amount of skin being
prepared. 1 ml. R.L. solution was used per g. skin. The solutions were applied to the blister area when convenient (immediately, to 1 hr. after preparation). Figure 60 (a) and (b) shows the pain-producing actions of rat and human skin extracts respectively on the cantharidin blister area (-subjects J.W.M. and D.E.A.) The pain, of moderate to severe intensity, was delayed in onset, slow mounting and long lasting. Itch usually occurred but this was after a longer delay (over 2 min). The pain was not due to the histamine content of the solution as this was assayed (by Dr. Franz Hobbiger) and found to be of the order of $5 \times 10^{-6} \text{g/ml. extract}$ (see Table 3, p. 79). However, the histamine is probably responsible for the itching which occurred.
2. **SUPPLEMENTARY BIOLOGICAL TECHNIQUES** - their Nature and Properties and certain Results obtained from them regarding the Stability of the Pain-producing Substance of Plasma and Inflammatory Exudates.

At this stage in the research, progress was considerably aided by the development of supplementary techniques, using excised animal tissues. The following test-preparations were used,

A. isolated rat uterus,
B. isolated rabbit-intestine,
C. isolated guinea-pig gut,

and it was found that pain-producing activity in blood and exudates was accompanied by parallel smooth-muscle stimulating activity. The advantages of using such an isolated organ preparation are that:

i. It is convenient to set up.

ii. Fluids of pathological origin can be used without danger.

iii. Fluids which markedly depress the blister area can be administered frequently.

iv. One such preparation, viz. the isolated rat uterus, is extremely sensitive to the active agents of serum, plasma and inflammatory exudates and shows steep dose-response relationships over suitably wide ranges of
concentrations.
The effective discriminating range of the isolated tissue can be found rapidly and conveniently by varying the volume administered.

v. Unlike the blister area technique, test doses may be varied by change in administered volume. This may be the only expedient method of testing and varying the dose given of an activity present in a solution of which the properties, (i) in dilute form soon alter (cf. Schachter, 1955 and 1956), or of which (ii) the activity, in concentrated form, rapidly changes with respect to time. (see below). (Both changes occur in human plasma.)

vi. A whole experiment may soon be performed.

A. Isolated Rat Uterus Technique.

The rat uterus preparation was set up in the following way:-
The rat, weighing about 200 g, was killed by a blow on the head and the uterus exposed. About two thirds of the length of the organ (both horns) were used by attaching the cervical portion to the immersed hook of the organ-bath and the horns, about two thirds of each, cut to be of equal length, to the writing lever by means of a length of linen thread looped through them and attached, at a gentle stretch, to the lever. The tissue in a
5 or 30 ml. organ bath, at a temperature of 25 - 30°C, kept constant in any given experiment, was bathed in a solution of the following composition:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>g/ml.</th>
<th>% w/v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td></td>
<td>$9.2 \times 10^{-3}$</td>
<td>0.92</td>
</tr>
<tr>
<td>KCl</td>
<td></td>
<td>$4.2 \times 10^{-4}$</td>
<td>0.042</td>
</tr>
<tr>
<td>CaCl₂</td>
<td></td>
<td>$6 \times 10^{-5}$</td>
<td>0.006</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>$10^{-3}$</td>
<td>0.1</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td></td>
<td>to give pH of 7.5 to 8</td>
<td></td>
</tr>
</tbody>
</table>

and 'aerated' with O₂, 100%.

With the uterus in a 5 ml. bath, or larger, it was found to be unnecessary to pre-heat administered solutions or to adhere to strictly uniform volumes of administration. The time of onset of contraction, its height and configuration were noted.

The tissue responds by contraction, after a latent period, to blister fluids (cantharidin, burn, insect-bite), joint fluid (rheumatoid arthritis), pleural fluid, ascitic fluid (of infective origin), hydrocoele fluid, plasma, serum, aqueous extracts of rat and human skin, and 5-HT. Examples are shown in figure 61. The rat uterus does not respond to cardiac oedema fluid applied.
similarly. It is markedly insensitive to histamine and to $K^+$, thus acting as a differentiating organ between these and the chemicals under test (Fig. 62).

The rat uterus responses to these fluids of human origin are not foreign protein reactions since rat's blood and plasma behave similarly to human blood and plasma when applied to the rat uterus, (Armstrong, Jepson, Keele and Stewart, 1955), (Fig. 63).

Parallelism of Response of the Rat Uterus and the Pain Area.

The rat uterus is apparently responding to the same agents in the test fluids as is the blister area;

1. Fresh whole blood is inactive on both preparations - see Figs. 69 and 586.

2. Serum: - a. stimulates both tissues
   b. the activity is stable as measured on both tissues (and as compared with the labile materials to be mentioned)
   c. Chemical extraction, e.g. with acetone, which separates out the active principle for one preparation does so also for the other.

   a. When these fluids are collected into a glass syringe and tested when they are well under
an hour old they cause marked pain and uterine stimulation.

b. The activity is not stable and fluid tested long after collection shows greatly reduced or no activity on either preparation. Fig. 64a shows the decline in uterine stimulating activity of burn blister fluid which occurs along with the decay in pain stimulation shown in Fig. 53. It will be seen that the burn blister fluid five minutes after removal from cold storage produced very severe pain, and that at $t = 0$ and $t = 10$ minutes after removal from the cold it produced brisk contractions of the uterus. After 1½ hours at room temperature, 20°C, the solution did not produce pain, and on the uterus the activity is shown to decline steadily till a single dose of blister-fluid gave no response (50 min. here) whilst one dose straight from the deep freeze was as active as ever.

The fluid from a blister induced by an insect bite behaves in the same way, as shown in Figures 51(A) (pain) and 64(b) (uterus). Here a single dose applied to the uterus failed to elicit a response by 35 min. whilst once more, material from the deep freeze was still active.
The pain-producing and uterine stimulating activity of fluid from rheumatoid arthritic joints, pleural effusions, protein-containing ascitic fluids and hydrocoele fluid decays in the same way as does the activity of human plasma whence all these come.

See Figures as follows:

Decay of activity - burn blister fluid - Fig. 64(a)

insect-bite blister fluid - Fig. 64(b)

rheumatoid arthritic joint fluid - Fig. 65(a)

pleural effusion - Fig. 65(b)

human plasma - Fig. 66

(If increasing volumes of the decaying fluid be added to the uterus bath as time and decay proceed, the percentage decay of the uterine stimulating activity at different 'points' in time may be determined by the principle of finding the volume of fluid necessary to produce a given original effect. In practice, only one dose may be administered at a given point in time and results obtained take the form of 'more than' or 'less than' a certain percentage of original activity present at that time.)
c. Cardiac oedema fluid, collected and applied to the blister area and rat uterus preparations in the same way as the fluids in iii. a., stimulated neither tissue. (Fig. 67, pain)

d. Onset of activity in inflammatory exudates and in plasma:—'activation'

Examination of the first and last responses of Figure 64(a) and (b) suggest that, in the absence of other variables some activation of the blister-fluid may be taking place. The second responses of both tracings are also somewhat greater than the first. It would appear that activation of the fluid may be taking place in conjunction with the decay and that, following ten minutes exposure at room temperature, $20^\circ$C, after freezing, decay predominates. This hypothesis was tested on both the pain-area and the rat 'uterus preparations' and found to be the case, and the findings were of great importance for four reasons. They showed that:

1. the fluid as removed from a blister or inflamed site does not contain the pain-producing substance in active form.
2. Keeping in a glass syringe allows the pain-producing substance to form.
3. At a time when the blister fluid, or blood
is non pain-producing on the blister area, i.e. immediately after collection, it produces no contraction of the isolated rat-uterus. A few minutes later when it is powerfully pain-producing it also induces powerful contractions of the uterus.

4. The fact that the blood of various species (e.g. man, cat, guinea-pig, rat) is inactive, at least initially, when it is carefully collected and applied to the rat's uterus, again shows that there is some other factor involved than just reaction of tissue to foreign protein.

Figure 68 (a), pain and (b) uterus, illustrates onset of activity in fluid from a cantharidin blister. To carry out this experiment it was necessary for the subject to have two blisters - one, to provide the test-area which requires several minutes after opening to settle down before being suitable for receiving test solutions, the other to provide the autologous blister fluid absolutely fresh and ready for immediate application to the prepared blister base and to the awaiting isolated rat uterus.

It can be seen that though both preparations
were 'alive' neither responded to the fresh fluid applied at 20 sec. after inserting the needle of the aspirating syringe to the donor-blist er. However, when the fluid had been left in the glass syringe for some minutes it induced activity in both preparations. The marked uterine response which occurred when the test fluid was 6 minutes old is seen to have been induced by half the volume of fluid which previously caused no response at all. Blood, containing heparin or citrate as anticoagulant behaves in the same way, being inactive on both preparations on withdrawal from the body and rapidly acquiring pain and uterine stimulating properties. Fig. 69 (a) shows the lack of pain produced by a subject's own blood administered to the blister area at 2 minutes after inserting the needle into the subject's vein for collection of 20 ml. blood, and later the pain caused by his heparinised plasma at a total time of 7 mins. Fig. 69 (b) shows the actions on the rat's uterus of heparinised blood of the same age. As happened with the pain area, first of all there was no response, when the blood was fresh, and then at 7 minutes a marked response (uterine
contraction) was caused by the blood. Blood containing oxalate as anticoagulant has been tested on the uterus only and there was found to behave as for heparinised or citrated blood. The onset of pain-producing activity in citrated blood is illustrated in Figure 58b.

Fluid from burn blisters and rheumatoid arthritic joints was found to activate similarly to produce uterine stimulating and pain-producing activity. Cardiac oedema-fluid which also is inactive on withdrawal, remained so on both tissues even after ten minutes exposure to glass. Thus human inflammatory exudates and plasma do not produce pain or stimulate the rat uterus when first withdrawn from the body into a glass syringe. After a few minutes pain-producing and uterine-stimulating activities develop. Decay of activity takes place on both tissues and by one hour pain is no longer produced and a given low volume of solution once capable of inducing powerful uterine contractions is now no longer capable of evoking a response at all. Thus there is a parallelism between the two responses and the rat's
uterus is therefore responding either to
the pain-producing agent or to a substance
the life time-course of which follows a
similar course.
Complete parallelism between pain-producing
intensity and uterine stimulating activity
is not a thing which one would expect to
find as it would infer that both tissues
each have the same threshold for stimulation
by the pain-producing substance and that
the dose-response curves of the two tissues
for the pain-producing substance are the
same.
It is difficult to conceive, for example,
that two concentrations of a substance
producing say a given percentage difference
in response in terms of 'height' of
contraction of the rat's uterus would produce
the same percentage similar difference in
pain-intensity induced, and that in fact
does not occur. With suitable choice of
low volume of blister-fluid applied to the
uterus the uterine response to it may
disappear after half an hour or less (i.e.
100% difference in response) whilst the pain-
response over the same period may remain
near maximum. In the same way the time of
appearance of pain-producing substance in
the test solution (as measured by a contraction of the rat's uterus) may be varied by selection of the magnitude of the dose given to induce it. However, initial and late zero-levels of activity occur and, most important, the peak activities occur at the same time—about 5 - 10 minutes whilst, on either side of peak, the intensities of effect produced rise and fall together.

A technique was then devised whereby blood, plasma or inflammatory exudates, with or without anticoagulant, could be kept in a "pre-active" state for an hour or more after withdrawal from the body.

The technique of Preparation of "Pre-active" Fluids consists of:

1. Careful collection into a cooled siliconed syringe through a siliconed needle.

2. Transfer to and maintenance in polythene containers in the cool - 7°C.

If it is desired to centrifuge the fluid, it is done in polythene tubes at room temperature (20°C) and the supernatant is carefully transferred by a siliconed needle and syringe to storage polythene pots in melting ice.
Blood, plasma or inflammatory exudate carefully collected and stored in this way is inactive, or almost completely so, on either preparation and such inactive material I call "pre-active".
Activation of 'pre-active' human plasma to form P.P.S.
When 'pre-active' plasma, as described, is transferred to glass, left for a few minutes, and then applied to both tissues marked pain-production and uterine stimulation take place, respectively. I now describe the plasma as "active" (glass-activated plasma - g.a.p.). The glass activation of pre-active human plasma is not dependent upon the presence of platelets as plasmas spun till platelet poor (< 20/cu.m.m) activate in the usual way on exposure to glass.
Figure 70 shows the actions of pre-active human plasma and of the same plasma after seven minutes exposure to glass when applied to each preparation at the same time. It is shown that the pre-active plasma caused no pain during one minute's application to the blister area, and that during one and a half minutes after the administration of 0.2 ml. to the isolated rat uterus it gave
only a tiny contraction occurring at 55 sec. (note, the time of onset of contraction of the rat's uterus for a variety of delayed action substances, e.g. 5-HT, bradykinin, angiotonin, is inversely proportional to the amount given). In contrast the plasma exposed to glass produced delayed pain (delay 20 sec.) which gradually mounted to reach moderate intensity (2 units) during the one minute of application to the blister area; it also produced marked uterine contraction occurring at 16 sec. following application of only half the volume which previously had no effect.

The activation shown here for pre-active plasma on exposure to glass resembles in all ways the activation of plasma, or inflammatory exudate collected, directly into an ordinary un-siliconed glass syringe and left for a similar period of time (- see Figs. 68 and 69 ).

'Pre-active' inflammatory exudates and blood activate similarly on transfer to glass. Blood stored without anticoagulant will liberate also 5-HT.

The "pre-active" material stored in polythene pots, becomes active though only
very slowly as compared with the rate of activation in glass; and during the hour or more whilst it is still "pre-active" aliquots may be serially used in studies on the activating (and decay) system.

In the course of its glass-exposure pre-active plasma increases in pain-producing and uterine stimulating potency, and activity reaches a peak at a time which is related to the volume of plasma exposed to area of glass in such a way that the more complete the exposure the earlier the peak is obtained. It is apparently not a glass-air phenomenon since activity rapidly develops in plasma in polythene covered by liquid paraffin when glass powder is dropped through. On an average, peak activity is observed at between 5 and 10 minutes after exposure of these protein-containing body fluids to glass. It is such that 0.01 ml. added to a 5 ml. organ bath will cause contraction of the isolated rat-uterus and a five-fold dilution of the plasma made immediately before application, will cause severe pain on the blister area. Decay then rapidly supervenes as described for glass collected specimens and by 15-20 minutes of exposure of the plasma to the glass the activity has declined so that only
about 50% of peak activity is present at that time. Plasma in its state following peak activity till about 50% of peak activity I describe as 'post-peak plasma'. Plasma later in time and of lower activity I describe as 'decayed plasma'. Decay is such that by one hour after glass exposure only about 10 or 5% of peak activity is present, as measured by the inverse ratio of the large volume of fluid then required to elicit the equivalent of 'peak' contraction on the rat's uterus to the small volume previously required at the time of peak-activity.

Perhaps the most important observations regarding the responses of the pain area and rat uterus being induced by the same e. agents are those provided by — e. Observations on the parallelism of responses of the tissues following chemical extraction of the pain-producing agent, P.P.S. P.P.S. is extractable in boiling EtOH and extracts made from 'pre-active', glass-activated, and decayed plasma showed absent, high and poor activity on both preparations. Figure 71 (a) and (b) show respectively the pain and the uterine responses caused by extracts made, by two volumes of boiling
absolute EtOH, from plasma which had been exposed to glass for:

40 minutes - 'decayed'
Not at all - 'pre-active' plasma, 'p.a.p.'
7 minutes - 'g.a.p.'

It will be seen that the responses run in parallel on both tissues and that the extract of decayed plasma had a small effect, that of pre-active plasma had no effect. The extract of glass-activated plasma, however, produced delayed slowly mounting pain which reached almost severe intensity during its one minute application to the area, and induced good contraction of the rat's uterus at a dose-level one quarter of that given of the extracts from decayed and pre-active plasmas and which respectively induced little and no uterine contraction.

iv. Heated human plasma

When the actions of heated pre-active plasma (see iii. above) and those of pre-active plasma kept at 37° for the same length of time, were compared simultaneously on the pain-area and the rat's uterus comparative records such as Fig. 72 were obtained. It is shown that the heated plasma is both uterine stimulating and pain-producing whereas that kept at 37° stimulates neither preparation.
Conclusion

1. Human blood and plasma following certain types of 'damage' induces pain and stimulates the isolated rat's uterus. The effect on the uterus is not a species (foreign protein) one since rat's blood on the rat's uterus behaves in the same way as does human blood on the rat's uterus.

2. Fresh blood straight from the body does not stimulate either preparation.

3. Serum causes delayed long-lasting pain and contraction of the rat's uterus. The activity is stable.

4. When human cell-free plasma or inflammatory exudate is exposed to glass, onset and decay of activity on the pain area and on the rat uterus, have been shown to occur. The responses run in parallel.

5. Onset of stimulant activity for both preparations has been shown to occur in human plasma when it is heated.

I felt justified in continuing to use the rat uterus technique as an adjunct to pain production in these investigations.
B. Isolated Rabbit - Intestine.

The rabbit intestine was set up in the usual way and bathed in a solution of the following composition:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>% w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>$8 \times 10^{-3}$</td>
<td>0.8</td>
</tr>
<tr>
<td>KCl</td>
<td>$2 \times 10^{-4}$</td>
<td>0.02</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>$2 \times 10^{-4}$</td>
<td>0.02</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>$10^{-4}$</td>
<td>0.01</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>$10^{-3}$</td>
<td>0.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>$10^{-3}$</td>
<td>0.1</td>
</tr>
</tbody>
</table>

and the bath 'aerated' with a mixture of O$_2$ and CO$_2$ (95% : 5%). Bath temperature was 37°C.
The preparation responds (by initial inhibition of tone and activity, followed by a marked increase in these before return to 'baseline') to active blister fluid and plasma. The rabbit gut was indeed the first isolated organ preparation on which the unstable nature of the active principle of protein containing body fluids was demonstrated. Fig. 73 shows the typical effect of active cantharidin blister fluid on the rabbit gut when 0.05 ml. of the fluid was administered at 6 min. after withdrawal from the cantharidine blister into a glass syringe. In Fig. 74 are shown the responses of rabbit gut to cantharidine blister fluid of different ages, viz. 3 min. and 2 hr. When the fluid was 3 minutes old a marked effect was obtained using 0.1 ml. but at 2 hours no such response was obtained.

The rabbit gut also shows the transition of human plasma from 'pre-active' to 'active' and 'decayed' states.

I have used it also to confirm differences observed between the various end products of various chemical and physical procedures upon active plasma and inflammatory exudates applied to the rat uterus. For routine work I preferred to use the rat uterus test since the whole behaviour of the tissue and its responses is more clearly defined and the record more readily readable than those
of the rabbit gut. The rabbit gut may, however, be regarded as a helpful adjunct to rat uterus comparisons where chemical identity is in question.

C. Isolated Guinea-pig Ileum.

The guinea-pig ileum responds to human plasma called 'active' (by virtue of its pain and rat uterus stimulating power) by contraction after a latent period. It does not respond to plasma called pre-active in accordance with the tests described. The contraction is rather sluggish and poor. The responses of all three isolated tissues viz. the rat uterus, rabbit gut and guinea-pig ileum to active human plasma and also to bradykinin are very similar. During the Spring of 1955 I worked out the relative sensitivities of the guinea-pig ileum and rat uterus tissues to P.P.S.-like substances in collaboration with Dr. M. Rocha e Silva, Sao Paulo. In this section the guinea-pig ileum tracings were made by Dr. M. Rocha e Silva simultaneously with the corresponding ones on the rat uterus made by myself. Dr. Rocha e Silva's method of assay has been described (Rocha e Silva, 1952). There is correlation between the responses of the rat uterus and those of the guinea-pig ileum to applications of human plasma and inflammatory exudates.
Fig. 75 shows activation of human plasma after removal from the body (the silicone-polythene technique already described; whereby the blood or plasma, with or without anticoagulant, could be kept in pre-active state, was employed prior to the activation).

The absence of response of both tissues to plasma straight from polythene is shown Fig. 75, then transfer to glass and subsequent testing was accompanied by onset and decay of activity on both tissues.

Correlation of response of the guinea-pig ileum and rat uterus to rat plasma on similar activation is shown in Fig. 76 (a) and (b) respectively, and the decay of "rat activity" is shown in Fig. 77 (a) and (b) whilst the non-activation of the plasma of another species viz. rabbit, on the rat uterus is accompanied by non-activation on the guinea-pig ileum also, and is shown in Fig. 76.

Both tissues respond to bradykinin which produces similar responses to active human plasma on them (the pain-area also responds to bradykinin as for active exudate and plasma). Thus a definite parallelism exists between the guinea-pig ileum and rat uterus for stimulation by agents of this bradykinin-like type. The thresholds and slopes of the tissues varied,
Table 5.

Comparison of the Sensitivities of the Guinea-pig Ileum and Rat Uterus to Bovine Bradykinin.

<table>
<thead>
<tr>
<th>RAT UTERUS (R): 30ml. bath</th>
<th>GUINEA-PIG ILEUM (G): 5ml. bath.</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>15.4.55.</td>
<td>709</td>
</tr>
<tr>
<td>(a)</td>
<td></td>
</tr>
<tr>
<td>15.4.55.</td>
<td>656</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
</tr>
<tr>
<td>18.4.55.</td>
<td>647</td>
</tr>
<tr>
<td>29.4.55.</td>
<td>300</td>
</tr>
</tbody>
</table>

\[ \frac{\text{R/G}}{\text{W/G}} = \frac{525 \times 30}{52} = 60 \]
however, the rat uterus being considerably more sensitive than the guinea-pig ileum. Fig. 78 shows the responses of the two preparations to various doses of bovine bradykinin. The sensitivities of the guinea-pig ileum and rat uterus were compared using 4 experiments in 3 days on 3 preparations of each. The results are summarised in Table 5, where the responses on the rat uterus have been measured as:—

\[
\text{height of response} \times \frac{1}{\text{time of onset of response}}
\]

and those on the guinea-pig ileum as maximum height of response. (Time of onset of response of a 'slow-reacting' substance on the guinea-pig ileum is not critical). I then calculated the log-dose response curves according to the formula:

\[
y = \bar{y} + b(x - \bar{x})
\]

where \( y = \text{response} \)

\[
\bar{y} = \text{mean response}
\]

\[
b = \text{slope} = \frac{\sum y(x - \bar{x})}{\sum (x - \bar{x})^2}
\]

\[
x = \log \text{dose}
\]

\[
\bar{x} = \text{mean log dose}
\]

The great difference in the slopes of the log-dose response curves of the two preparations is at once apparent. The threshold doses are extrapolations of the log-dose curves and are found to be similar when used with these baths of 6/1 capacity (R/G).
I calculated the value
\[ \text{Slope}_R \times \text{Threshold}_R \times \text{Bath volume}_R \]
\[ \text{Slope}_G \times \text{Threshold}_G \times \text{Bath volume}_G \]

where \( R = \) Rat Uterus
\( G = \) Guinea-pig Ileum

and obtained the figure 60.

This indicates only that the rat uterus is a much more sensitive test organ for bradykinin, the threshold being much lower, (in terms of concentration in the bath fluid), and the slope of the dose-response curve much steeper.

In Conclusion.

A comparison of the guinea-pig ileum and the rat uterus preparations for the assay of P.P.S. and P.P.S.-like substances has been made. It was found that:-

1. The rat uterus technique has the following important properties:-

1. Ability to detect low concentrations of certain polypeptides e.g. bradykinin. One microgram of P.P.M. bovine bradykinin added to a 30 ml. bath, equivalent to a final concentration of \( 3 \times 10^{-8} \text{g/ml.} \) P.P.M. material (and less of active principle) will cause contraction, whereas the guinea-pig ileum requires tens of times this concentration.
The threshold for the rat uterus, measured in terms of human bradykinin, is equivalent to about 2\(\mu\)l. of human plasma and this represents a final concentration of human bradykinin, in the organ bath, equivalent to 0.07\(\mu\)l. parent plasma / ml. bath fluid. The guinea-pig ileum requires correspondingly higher concentrations.

ii. The responses have well-defined maximum height of contraction, for a given dose, since the tissue after fairly rapid contraction, once started, relaxes promptly thus clearly defining the limit. This is in comparison with the sluggish responses of the guinea-pig ileum.

iii. The increase in height of the rat uterine response and the decrease in latent period before onset of contraction for small increase in dosage is marked i.e. there is a steep slope to the dose-response curve. The dose-response curves of the guinea-pig ileum for bradykinin and active plasma are not steep.

iv. The rat uterus is not sensitive to histamine and potassium ion, two common contaminants or interfering substances in materials of biological origin. At least 1 mg of either of these agents must be added to a 30 ml. organ bath to elicit a response.
Unlike the guinea-pig ileum which is depressed by administrations of human plasma the rat uterus maintains its high sensitivity to active human plasma over prolonged periods of time.

2. The guinea-pig ileum and rat uterus respond in the same way to

a. human plasma during onset and decay of the plasma pain-producing substance. A similar parallelism exists regarding the responses of the two tissues to

b. the labile substance of glass exposed animal plasmas when this occurs, e.g. in the rat and guinea-pig.

c. Those plasmas which are not similarly activated, as shown on the rat uterus, (e.g. those of rabbit and dog) are also inactive on the guinea-pig ileum.

3. Just as I found the rat uterus superior to the rabbit gut for routine testing of the plasma substance so I prefer it to the guinea-pig ileum for the reasons given.

4. The guinea-pig ileum could be of use in circumstances where bradykinin-like substances, in widely differing concentration, are to be tested without prior dilution and where high concentrations of material are available in small volume. Though the concentration of
P.P.S. in human plasma varies manifold in the course of its formation and decay, in its initial and late stages the levels are below the threshold of the guinea-pig ileum for detection.

5. Unlike the use of the rabbit gut the use of the guinea-pig ileum has furnished no further information regarding the properties of P.P.S. (except that the guinea-pig ileum is not very sensitive to it) and I felt satisfied that the continued use of the isolated rat-uterus technique in the investigation of the pain-producing materials of biological origin was worth-while since it is the most sensitive, and discriminating of the three isolated preparations examined. Furthermore it is very sensitive to other polypeptides besides bradykinin (e.g. angiotenin, oxytocin) and may be used to distinguish between them. (see p. 140).
3. EXPERIMENTS CARRIED OUT ON THE CANTHARIDIN BLISTER AREA AND ISOLATED RAT UTERUS USING THE FLUIDS OF PATHOLOGICAL ORIGIN AND/OR CHEMICAL DERIVATIVES FROM THEM, IN AN ATTEMPT TO ELUCIDATE THEIR CHEMICAL NATURE - further investigations on the production of pain by:

a. human inflammatory exudates and plasma  
b. 'damaged' human blood  
c. skin extracts.

da. Human Inflammatory Exudates and Plasma.
Since the pain-producing principle of 'active' human plasma is unstable like that of the inflammatory exudates, and since plasma is in fact the origin of the inflammatory exudates, it is classified with them in the subsequent investigations.

1. Relationship of the Active Principle to:

1. Acetylcholine. The delayed slow-mounting, long maintained pain elicited by P.P.S. on the blister area in no way resembles the actions of acetylcholine on the area (p. 45). The uterine stimulating action is not antagonised by atropine. Fig. 79 shows burn blister fluid acting on the uterus in low dosage which would be equivalent, in terms of ACh, to 50 mg/ml. which is apparently absurd.

11. Histamine.  }

111. Potassium Ion. 
The pain-producing actions of histamine and of K⁺ have been described (pp. 79 and 77 respectively) and these differ from the actions of P.P.S. on the blister area (p. 100). The rat uterus is not sensitive to these substances and at least several mg must be added to the uterus bath to elicit contraction; on the other hand the uterus is very sensitive to P.P.S. and 0.01 ml. of plasma at peak activity will elicit a contraction. Thus in terms of histamine or potassium ion P.P.S. activity would be equivalent to several hundred mg/ml. in 'peak' plasma. It is obviously not profitable to further pursue these relationships since P.P.S. is therefore apparently neither histamine nor potassium.

iv. Adenosine triphosphate.

ATP is not a potent stimulating agent for either tissue (i.e. pain area or rat uterus) since $10^{-4}$g/ml., or $10^{-4}$g, respectively, does not elicit pronounced responses. P.P.S. at peak activity is markedly painful and oxytocic and equivalent concentrations of ATP once more work out at several mg/ml. plasma — Fig. 80. The same figure shows also that the dose-response relationships of the rat uterus for ATP and for P.P.S. of the fluid
from a blister induced by an insect bite, are different. The active principle, P.P.S., is thus apparently not ATP.

v. 5-hydroxytryptamine.

Though 5-hydroxytryptamine elicits the same type of pain as P.P.S., after its application the blister area is markedly refractory to it (5-HT). This is not the case following P.P.S. applications. 5-hydroxytryptamine is stable in plasma, P.P.S. is not. Fig. 81 shows decay of activity of P.P.S. in burn blister fluid whilst the activity of 5-HT added to another aliquot of the same fluid remained. Though the dose of 5-HT administered in the mixture was only a 'threshold' one the response was unchanged after the P.P.S activity of the same volume of plasma had disappeared.

5-HT added to plasma is extracted by 95% acetone; plasma P.P.S. activity comes down with the precipitated proteins. Figure 82 (a), shows the response of the rat uterus to 0.25 ml. of acetone precipitate of active burn blister fluid and absence of response to 0.75 ml. of extract. The activity of the precipitate, like that of the parent fluid, was unstable at room temperature, 20°C.

Fig. 82 (b). When, however, 5-HT was added
to another aliquot of the same burn blister fluid and this was similarly treated with acetone the extraction was almost quantitative Fig. 83 (a), and the extract was stable - Fig. 83, (b).

The actions of 5-HT on the rat uterus are antagonised by dihydroergotamine, (D.H.E.), and by lysergic acid diethylamide, (L.S.D.) (Gaddum and Hameed, 1954) those of P.P.S. are not - see Figs. 84 and 85.

P.P.S., must be regarded as some substance other than ACh, histamine, potassium, ATP or 5-HT.

2. Isolation of pain-producing, uterine stimulating Activity from Active Human Plasma and Inflammatory Exudates by Extraction and by Ultrafiltration: Properties of the Products.

The active principle of glass-activated plasma is heat stable. It can be boiled in R.L. solution without loss of potency during several hours. The activity is extractable in R.L. solution, and also in boiling ethyl alcohol. The extracts are stable. The activities of the extracts closely resemble those of the parent materials. Figure 71 shows the actions of ethyl alcohol extracts of 'pre-active', 'active' and 'decayed' plasma on the pain area and on the rat uterus.
The activity of plasma and inflammatory exudates is **ultrafiltrable** and the ultrafiltrate is 'stable'. Figure 86 shows actions on the uterus of a sample of ultrafiltrate, made in the cold, under pressure, of my own plasma, taken half way through its course of filtration i.e. at about 6 hr. old. The uterine responses to P.P.S. closely resemble those to bovine bradykinin (though the relationship of time of onset of response to height of contraction is not quite the same as with the bradykinin responses); Substance P had no effect on the tissue.

Figure 87 shows the pain produced by ultrafiltrates of burn blister fluid and of active human plasma.

The potency of the ultrafiltrates for pain production and uterine stimulation is always low, being of the order of one fifth to one quarter that of plasma at peak activity. The low activity of the ultrafiltrate may be attributed to decay whilst in the ultrafiltration apparatus.

The ultrafiltrate itself is relatively stable at R.T., resists boiling in N/3 HCl for 10 min., but is largely destroyed by boiling in N/3 NaOH for 10 min.

Freeze-dried ultrafiltrate was used to study
solubility properties. The ultrafiltrate is soluble in (a) 85% ethyl alcohol, but not in absolute alcohol (Fig. 88); (b) it is soluble in hot methyl alcohol.

Unlike the activity in parent glass-activated human plasma, which is precipitated with the proteins and not extracted by acetone, (Fig. 89(a)) the activity after ultrafiltration is soluble in 90% acetone, although it is not soluble in dry acetone. Fig. 89(b). In the presence of protein the ultrafiltrate activity is precipitated by acetone, as in the parent plasma, Fig. 90.

In the presence of added protein ultrafiltrate activity can be extracted by glacial acetic acid plus ether (Fig. 91).

The active agent in an ultrafiltrate of active human plasma can be absorbed by charcoal and by cellulose but not by barium sulphate. The activity can be removed by ion exchangers, the removal being almost complete with cation exchange (H⁺ form) and partial with anion exchangers (OH⁻ form).

3. **Enzymatic Digestion of Ultrafiltrate and Ethyl Alcohol Extract of Active Human Plasma.**

The ultrafiltrate and ethyl alcohol extracts of human plasma are rapidly digested by human plasma.
The ultrafiltrate however, differs markedly in its potency and in its enzymatic digestion responses from an EtOH extract made from plasma at its peak activity. The ultrafiltrate is about one fifth as potent as the extract and is easily digested by chymotrypsin (Fig. 92), whereas equiactive EtOH extract (i.e. equioxoytocic) is only poorly digested. It is concluded from these and the solubility tests that the active principle of ultrafiltrate of human plasma is peptide in nature. Its low potency is attributed to the time taken for its production (8 - 12 hr.) during which period the pain-producing activity of plasma would be decreasing. It is thus possible that an ultrafiltrate of human plasma is rich in less active degradation products of the chemical predominant at the time of peak activity. The EtOH extract of active human plasma though not digested by chymotrypsin is digested by amino-peptidase from E. Coli, and hence it also is peptide in nature. Volume for volume the activity in the extract closely represents the activity of parent 'peak' plasma whilst, as already shown, pre-peak and post-peak EtOH extractions are correspondingly less active. It would seem therefore that EtOH extract represents the most active state of human
glass-activated plasma and that this active principle is polypeptide in nature.

4. The Nature of the Activation and Decay Processes in Human Plasma.

i. The pain-producing and uterine stimulating activities of human plasma exposed to glass are rapid in onset and in decay.

ii. Activity is not dependent on the presence of cells since cell-free 'pre-active' plasma activates in the usual way.

iii. The soya bean trypsin inhibitor (S.B.T.I.), which has no effect on the rat uterus response to formed P.P.S. - inhibits the onset of P.P.S. activity when 'pre-active' human (or rat) plasma is put into glass - Fig. 93. This suggests that the formation system is a proteolytic one.

iv. Disodium Versenate - disodium ethylenediamine tetra acetic acid.

When disodium versenate is premixed with plasma transferred to glass it has no inhibiting action on formation of P.P.S. When it is added to glass-activated plasma it greatly slows decay. Fig. 94 shows the effects of adding versene, 1 mg/ml. to some plasma after 7' exposure to glass and keeping the mixture, and a control (versene-free), at room temperature (20°C). One and a
quarter hours afterwards the vesene sample was still active, whereas the control sample had lost its activity in half that time. The above findings suggest that there are two systems responsible for the behaviour of plasma in glass - one for onset of activity (a proteolytic digestive process yielding active pain-producing and uterine stimulating polypeptide; inhibited by S.B.T.I.) and another (metal-requiring proteolytic system) responsible for the decay. Both are heat labile. The destroying system is present from the beginning (i.e. does not require to be glass activated) since plasma in polythene will readily destroy EtOH extract of glass activated plasma.

Conclusions:

i. When fresh human plasma is exposed to glass a pain-producing, uterine-stimulating substance arises.

ii. The active agent is polypeptide in nature and is extractable in R.L. and EtOH solution.

iii. Activity can also be ultrafiltered from active plasma but the process is slow and activity is low.
iv. Extraction or ultrafiltration of the pain-producing polypeptides from the parent plasma stabilises these substances.

v. Apparently two systems are present in plasma and are active in the life-course of P.P.S. - one, proteolytic, responsible for development of the pain-producing and uterine-stimulating properties. It requires an activator such as glass to arouse it to high activity leading to rapid, full development of P.P.S. The other system, responsible for decay, is probably a peptidase and is always highly active independent of glass contact. It apparently requires metal for its activity.

b. 'Damaged' Human Blood.

i. Serum.

ii. Plasma.

iii. Heated plasma.

i. Serum.

The production of pain and uterine stimulation by serum is probably due to 5-hydroxytryptamine for the following reasons:

1. 5-hydroxytryptamine produces pain and stimulates the rat's uterus in the same way as does human serum.

2. The activity of serum is stable and extractable in acetone. This resembles
the actions of 5-HT in serum or plasma and differs from the P.P.S. activity of plasma which is not stable and which is precipitated by acetone. The extract of serum is likewise stable.

3. The actions of serum, and of its acetone extract, on the rat uterus are easily antagonised by dihydroergotamine.

4. 5-HT occurs in serum and platelet extracts (see Part III, 1) in quantities sufficient to produce marked pain, i.e. over $10^{-7}$g/ml., such as occurs when serum is applied to the raw test-area.

ii. Plasma — see Part III, 3a above.

iii. Heated human plasma.

When 'pre-active' heparinised human plasma is heated to 70-75°C in polythene vessels it develops pain-producing, uterine stimulating activity (Fig. 72). In some experiments the plasma was heated by passing it rapidly through heated thin-walled polythene coils and Fig. 95 shows activity which occurred during a passage lasting only 10 sec. (It is also shown that the mere passage of the plasma through the coils at 37°C does not activate the plasma). In these experiments cell-rich (i.e. platelet-rich) plasma was more readily activated than cell-poor plasma (Fig. 96). This suggests
that the active agent might come from platelets and might therefore be 5-HT. In agreement with this view are the following findings:-

1. Heated plasma applied to the blister area causes pronounced pain of a type caused by 5-HT. (Fig. 72).

2. The substance is stable in plasma (Fig. 96).

3. The action on the rat uterus resembles the actions of 5-HT on that tissue and is antagonised by dihydroergotamine (Fig. 96).

4. The activity is acetone-soluble. When extracted from plasma, in 95% acetone more than 80% of the activity was found in the extract (Fig. 97). This activity, measured in terms of 5-HT was equivalent to $10^{-6} \text{g/ml.}$ in the cell-rich plasma.

In a few experiments heated plasma contained an unstable uterine-stimulating substance (Fig. 98).

Conclusion.

Cell-rich human plasma, when heated to 70-80°C in polythene vessels, rapidly develops a pain-producing, uterine stimulating substance resembling 5-hydroxytryptamine in its actions.

C. Skin Extracts.

The work on skin extracts has been mainly on
rat skin as this is more readily available than human skin.

The skin was extracted in R.L. solution as described on p. 107 and when applied to the blister area caused marked pain of delayed, long-lasting type, and some itching (see p. 108). Applied to the rat's uterus the extracts caused contraction. The extract was unstable.

The following agents are excluded from being responsible for the pain caused by skin extracts:

1. **Histamine.**

   Histamine does not occur in the skin extracts in concentrations high enough to account for the pain produced (see p. 108 and Table 3) though it is probably responsible for the itching which occurs (p. 108). Histamine does not contract the rat's uterus.

2. **Acetylcholine.**

   The type of pain produced by skin extract does not resemble that produced by acetylcholine. On the rat's uterus the active principle in the skin extract differs from ACh in being much less antagonised by atropine (Fig. 99).

3. **5-Hydroxytryptamine.**

   Though the pain and the uterine responses to skin extract resemble those to 5-HT, and though the activity is acetone-soluble and ultra-filterable, concentrations of dihydroergotamine
which antagonise 5-HT on the rat uterus do not antagonise the action of skin extract nor its ultrafiltrate (Fig. 100 (a) and (b) ). The extracts are not stable when maintained at 37° (Fig. 101 ) but are rather more stable than rat or human P.P.S. in plasma. The ultrafiltrate is digested by plasma. (Fig. 102 ).

The skin extracts can be rendered protein-free also by boiling for 2 or 3 minutes in a slightly acid medium (pH 6.5).

Incubation of ultrafiltrate or protein-free extract with trypsin, chymotrypsin or the amino peptidase of E. Coli failed to remove the activity.

The activity of the extract is not prevented by the presence of the soya bean trypsin inhibitor in the extraction fluid nor is the duration of activity of skin extract in R.L. solution prolonged by disodium versenate as is the activity of P.P.S. in plasma.

Acetone extract of skin extract is markedly pain-producing, unlike the acetone precipitate. The extract causes contraction of the rat's uterus whereas the precipitate does not (Fig. 103 ). The active agent in an ultrafiltrate of skin extract is likewise acetone soluble. Even in the presence of added
protein acetone extracts the activity of the ultrafiltrate (Fig. 104).

The activity is soluble in ethyl alcohol whether protein is present or not. It is also soluble in diethyl ether. At pH 7.8 I found distribution of activity between the ether and the aqueous phases. At pH 2 however the activity left the aqueous phase and was found in the ether phase.

Activity of protein-free rat skin extract was largely destroyed by boiling in an acid medium (HCl, pH 2 approx.) whereas a control boiled in alkaline medium (Na HCO₃ pH 7.8 approx.) for the same length of time, 20 min., maintained its activity.

Conclusion:

The active agent in skin extracts is not histamine, acetylcholine or 5-hydroxytryptamine. The presence of the soya bean trypsin inhibitor in the fluid used to extract the skin does not prevent its activity. In this and in other respects the activity differs from that produced by dilution of plasma. (cf. Schachter, 1956). The resistance of skin extract to digestion by trypsin, chymotrypsin and the amino peptidase of E. Coli, along with its solubility properties and its ready destruction by boiling in an acid
medium indicate that the agent is not polypeptide. The properties are consistent with those of an organic acid such as Darmstoff (Vogt, 1949, 1955 and 1956) or the acid found! skin by Ambache and West (1956). Indeed, Darmstoff applied to the exposed blister base in fairly low concentration, e.g. $10^{-5}$ g/ml. produced pain of delayed and prolonged type such as occurs with skin extracts.

4. SUMMARY

a. Pain-producing agents have been shown to occur:
   i. when human plasma or inflammatory exudates are exposed to glass;
   ii. when blood clots, in serum;
   iii. when human cell-rich plasma is exposed to heat (55°C and above).
   iv. in aqueous extracts of skin.

b. The natures of the responsible agents are apparently as follows:
   i. Human plasma or inflammatory exudates — Polypeptide. This conclusion has been reached in view of:
      1. The pain produced by 'active' human plasma or inflammatory exudate is of the delayed, prolonged type such as is produced by 5-hydroxytryptamine or by polypeptides. The uterine responses agree with this. They
are not antagonised by dihydroergotamine, suggesting that the active agent is not 5-HT, and, moreover, the time-response pictures produced by the active plasmas or exudates on the uterus are more in keeping with the active agent's being polypeptide than 5-hydroxytryptamine or various other oxytocic agents (such as KCl, ACh).

2. solubility properties,

3. inhibition of formation in the presence of the soya bean trypsin inhibitor.

4. removal of P.P.S. activity by plasma, by chymotrypsin and by the amino peptidase of E. Coli.

11. Serum - 5-hydroxytryptamine, since

1. 5-HT is found in serum in concentrations which, when applied to the blister area produce pronounced pain of the type elicited by serum,

2. both serum and 5-hydroxytryptamine produce similar progressive depression of the blister area to themselves, to one another but not to other pain stimulants, e.g. acetylcholine,

3. on the rat uterus the response resembles that to 5-HT, and its actions are antagonised by dihydroergotamine.

iii. Heated 'cell-rich' human plasma - 5-hydroxytryptamine, since:
1. applied to the blister area the solution produced delayed, prolonged pain.
2. the activity on the rat uterus was antagonised by dihydroergotamine and was largely extracted by 95% acetone.
3. The activity arose in platelet-rich plasma but not in platelet-free plasma.

iv. Aqueous extract of macerated skin - possibly of Organic Acid nature since:
1. It could not be
   a. identified with such known agents as histamine, acetylcholine, 5-hydroxytryptamine, or
   b. destroyed by peptidases,
2. It is unstable on boiling in acid,
3. It is soluble in acetone even in the presence of protein, and distributes itself into the ether phase when shaken in acidified aqueous solution along with ether.
4. Material of this type has been found in aqueous extracts of skin (Ambache and West, 1956) and other tissues, Darmstoff (Vogt, 1949, 1955 and 1956).

5. HEAT BURN OF THE FOREARM.

Figure 105 (a) and (b) shows records of the pain experienced by two subjects during and after application of a hot rod to the forearm. The rod, at temperatures of 65°C and 70°C for the respective
subjects (D.A. and C.A.K.) was applied for 3 sec. in both cases. The records show that application of the hot rod produced immediate intense pain and following removal of the hot rod the pain subsided to zero very rapidly. There was then a period during which no pain was experienced, 27 and 21 sec. respectively. After this delay period pain appeared and slowly built up to reach a well-maintained peak. Then, after several to many minutes the pain gradually subsided.

Comment.

These observations regarding the initial and delayed effects of heat on the skin agree well with those obtained by Sir Thomas Lewis following burning of the skin with wax. 'The application is painful. Pain as an after effect may begin at once but often an interval of 10 to 30 sec. or even a minute after the withdrawal of the wax passes without pain. In about 10 min. the skin is red, a little swollen, and tender and is giving constant burning pain; this state continues for many minutes, an hour, or more' (Lewis, 1942). (Underlining mine). The very rapid onset and decline of pain-intensity which I have demonstrated to occur at the respective times of application and removal of the hot rod suggest that the stimulus for this pain may well be the direct one of heat. As for the ensuing, delayed pain, since there is no applied
thermal or mechanical stimulus to account for it, it would seem that it must result from some internal chemical or physico-chemical activity. 5-hydroxytryptamine may well be liberated from blood in the heated capillaries as it is from platelet-rich plasma heated in thin-walled polythene tubes. Proteolytic enzymes such as, for example, the heat-stable protease found in skin of man, the rat and other animals (Beloff and Peters, 1944 and 1945) may well be liberated from injured cells to produce polypeptides and cause pain. Cullumbine and Rydon (1946) have shown that the enzyme preparation of Beloff and Peters liberates from blood fibrin (but not blood albumin) a substance resembling leukotaxine (Menkin, 1937) and this type of substance has been shown to produce pain (this thesis, p. 94). Furthermore, Kellaway and Rawlinson (1944), by perfusion experiments on the cat's hind limb, demonstrated the setting-free of proteolytic enzymes by perfusion fluid of temperature 41°C and above and the output appeared to increase with the temperature of the perfusion. Pain-producing substances of organic acid nature as shown to be present in skin extracts (thesis p. 150), and histamine found in perfusates from heated limbs of cat and guinea-pig (Kellaway and Rawlinson, 1944), may also make their contribution. It might be expected that histamine and 5-HT would be liberated immediately by the heat,
and that other substances e.g. polypeptides and perhaps the organic acids might become active only after an interval. The delayed pain of a thermal burn might well be due to these agents.
GENERAL CONCLUSIONS.
GENERAL CONCLUSIONS.

1. A new technique for use in the study of pain has been developed and described. It involves stimulation of pain nerves without change in the temperature or pressure of their environment. Test solutions are applied to the exposed base of a blister induced by cantharidin. The blistering technique denudes down to the basal layer of the epidermis. No good reason has been found to suggest that the preparation is abnormally sensitive. Subjects make permanent graphic records of their pain, using a scale calibrated to indicate:

   no pain (= 0),
   slight (= 1),
   moderate (= 2),
   severe pain (= 3), and
   very severe pain (= 4),

the records themselves being invisible to the subjects during the course of the experiment.

2. The pain-producing properties of many described substances and preparations have been investigated.

3. Pain-producing properties of human blood, plasma and inflammatory exudates, and skin extract have been revealed and the agents responsible for the pain have been characterised.

4. In studying these pain-producing agents the use of certain isolated organ preparations (particularly
the isolated rat uterus) whose responses run parallel with those of the human blister area has been convenient and time-saving. This permitted the free use of pathological materials and also allowed the effects of chemical and physical treatments of the pain-producing agents to be rapidly evaluated. In this way it was possible to reduce the number of experiments which had to be performed on the blister area.

5. The cantharidin blister area technique is valuable in the study of antagonism both towards chemically and mechanically induced pain (see thesis, and Mongar, 1955, respectively).

6. Using the blister area technique different subjects give comparable results, as does the same subject on different days with different blister areas. The pain elicited varies in time of onset, rate of increase in intensity, duration of peak-pain and rate of decline, among different types of chemical agents. In general there are three main types of pain produced by substances in concentrations eliciting pain of moderate intensity:— (i.) sharp, 'needle-like' pain, rapid in onset and decline, e.g. that produced by acetylcholine, carbachol, nicotine, (ii) pain which is slightly delayed in onset (say 2-7 sec) and longer in duration, for the same intensity of pain, e.g. that to K⁺ and to histamine, (iii) pain of long delay in onset (say 15-45 sec or more), slow build-up, and
<table>
<thead>
<tr>
<th>AGENT</th>
<th>at which PAIN is NOT usually PRODUCED</th>
<th>at which PAIN is regularly PRODUCED</th>
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<tbody>
<tr>
<td>Tryptamine hydrochloride</td>
<td>$5 \times 10^{-8} \text{ g/ml.}$</td>
<td>$10^{-7} \text{ to } 10^{-6} \text{ g/ml.}$</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>$10^{-3} \ &quot; $</td>
<td>$-$</td>
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<tr>
<td>Bradykinin Preparation</td>
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<td>$10^{-5} \text{ to } 10^{-4} \text{ g/ml.}$</td>
</tr>
<tr>
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<td>$10^{-6} \ &quot; $</td>
<td>$10^{-5} \text{ to } 10^{-4} \text{ g/ml.}$</td>
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<tr>
<td>Hypertensin</td>
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<td>$10^{-4} \text{ g/ml.}$</td>
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<tr>
<td>'Pitressin' (Parke Davis)</td>
<td>$?$</td>
<td>$1 \text{ unit/ml.$(\approx}$ say, $10^{-6} \text{ g/ml}$)</td>
</tr>
<tr>
<td>AGENT</td>
<td>CONCENTRATION at which PAIN is NOT usually PRODUCED</td>
<td>CONCENTRATION at which PAIN is regularly PRODUCED</td>
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<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------------</td>
<td>----------------------------------------------------</td>
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<td>Tonicity</td>
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</tr>
<tr>
<td>pH</td>
<td>$&gt;3$</td>
<td>$&lt;3$</td>
</tr>
<tr>
<td>KCl</td>
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<td>$3 \times 10^{-3}$ g/ml.</td>
</tr>
<tr>
<td>ATP disodium salt</td>
<td>$10^{-6}$</td>
<td>$10^{-4}$</td>
</tr>
<tr>
<td>ACh chloride</td>
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<tr>
<td>Pilocarpine nitrate</td>
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<td>-</td>
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<td>5-hydroxytryptamine creatinine sulphate</td>
<td>$5 \times 10^{-9}$</td>
<td>$10^{-7}$</td>
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continued on previous page.
prolonged maintenance, e.g. that to 5-HT, serum and 'active' plasma.

The blister area responses to different agents are listed in Table 6. The responses to the polypeptides are to the various crude preparations described in the text.

7. The results with K⁺ (see p. 51) suggest that nerve fibres as well as nerve endings are exposed and can respond to applied chemicals.

8. The nerve endings are stimulated by acetylcholine, carbachol and nicotine but not by methacholine or by pilocarpine. They are readily antagonised in their response to acetylcholine by low concentrations of d-tubocurarine and decamethonium. High concentrations of atropine and hexamethonium are required to antagonize this action of ACh. The nerve endings thus have properties in common with the motor end-plate of voluntary muscle.

9. Adrenaline, nor-adrenaline, histamine and adenosine triphosphate are not potent producers of pain when applied to the blister area.

10. 5-hydroxytryptamine is the most potent pain-producing substance of known composition. It is found in human serum where it can occur in concentrations equivalent to 10⁻⁷g/ml. free base (see Borrelli & Zucker, 1955). "Significant" pain is produced by concentrations of 5 x 10⁻⁹g/ml., and above, of 5-HT base applied to the test area. Experiments described
in the thesis show it to have an 'optimal structure' for pain-production.

5-hydroxytryptamine is present in the dried venom of the common wasp, *Vespa Vulgaris*. The acute pain following wasp-sting is familiar. Only recently has it been possible to ascribe an accurate chemical basis for the induced pain. In 1953 the pain-producing actions of histamine were confirmed and evaluated, and the potent action of 5-hydroxytryptamine was demonstrated (Armstrong, Dry et al., 1953). In the following year the pain-producing actions of bradykinin and other polypeptide preparations were reported (Armstrong, Jepson et al., 1954). In the same year, 1954, Jaques and Schachter identified two of the substances, viz. histamine and 5-hydroxytryptamine, in high concentration in the dried venom of the wasp - the concentrations were equivalent to 4 and 6 micrograms, respectively, per venom-apparatus which would represent very high concentrations of the active agents in the small volume of injected sting-fluid. The high concentration of histamine would, in addition, aid dispersal and diffusion of the large amounts of 5-HT. A third substance was also found in the extracts, and designated 'kinin' (Schachter & Thain, 1954). It was a bradykinin-like substance producing delayed slow-contraction of the guinea-pig ileum and so might well have been expected to
contribute to the pain picture. Two years later application to the human blister base of a partially purified preparation of this kinin was reported to be indeed pain-producing and to represent a potency about ten times as great as acetylcholine (Holdstock, Mathias & Schachter, 1956).

Through the work of Emmelin and Feldberg, (1947b) who found acetylcholine, in $10^{-2}\text{g/ml.}$, and histamine, in $10^{-3}\text{g/ml.}$ concentration, in the hair-fluid of stinging-nettles (Urtica urens), and also a third substance, later characterised as 5-hydroxytryptamine in concentration of $4 \times 10^{-9}\text{g/sting}$ (Chesher & Collier, 1955) (equivalent, on the basis of hair-fluid weight [Emmelin & Feldberg, 1947a] to about $5 \times 10^{-7}\text{g/ml.}$ 5-HT in the sting fluid), and the work described in the thesis on the evaluation of the pain-producing properties of these substances, the mechanism of pain-production by nettles has become known and more fully understood. The interesting idea of a possible relationship of solanain (see Arthur and Shelley, 1955; and Greenberg and Winnick, 1940 a and b), a proteolytic enzyme found in nettles, to polypeptide and pain-production, and its relationship to the prolonged pain of nettle stings, is yet to be investigated.

The hair-like structures of the pods of macuna pruriens produce pain and itch when introduced to the human skin. They have been found to contain a substance
which "resembles others present in some snake venoms and also in bee venom, which have been shown to liberate histamine from animal tissues" (Broadbent, 1953), a proteolytic enzyme, mucunain, (Arthur & Shelley, 1955), and 5-HT in high concentration, $1.5 \times 10^{-4} g/g$ (Batty, Bowden & Brown, 1954).

11. The blister area experiments on the relation of toxicity and acidity of solutions to pain-production explains the very painful effects caused by contact of 'fresh' water, salt or sea-water, and acid (e.g. citric, acetic) solutions with wounds.

12. The pain-producing agents of human origin which have been examined have been characterised as follows:
   i. serum - 5-hydroxytryptamine,
   ii. 'active' plasma and inflammatory exudate - polypeptide,
   iii. heated platelet-rich plasma - 5-hydroxytryptamine,
   iv. aqueous extract of macerated skin - possibly organic acid.

13. Heat-burn of the forearm is described and evidence is put forward suggesting that the delayed pain of thermal injury to the skin may well be due to liberation of the substances described in 12 above.
APPENDIX.
ACKNOWLEDGEMENTS.
ACKNOWLEDGEMENTS

I wish to thank the Middlesex Hospital Medical School for providing me with the facilities for doing this work.

My most grateful thanks are due to Professor C. A. Keele whose willingness and ability to supervise the research I greatly appreciate, and whose inspiration to use the exposed cantharidin blister base as a test-area forms the corner-stone of the research.

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My most grateful thanks are due to all those Members of my Department, Students and Friends who trustingly submitted to prickings, bleedings and blisterings on behalf of the research. Without their co-operation the whole project would hardly have been possible.

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Lysergic acid diethylamide – Professor J.H. Gaddum,
Posterior-Root Extract – Mrs. P. Holton,
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(Roche Products Limited), Professor J.H. Gaddum,
Dr. E.K. Richards (Abbott Laboratories Limited).
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assistance.

For kindly drawing Figure 1, the pain-recording
apparatus, I thank Miss Jean Oswald.
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TABLES.

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Graph.


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105. Pain.
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Figure 1.

Apparatus for Recording Pain and Itch.

Compression of bulb, B, by turning screw-handle, H, forces mercury in manometer, M, up its distal limb to raise a pointer-bearing float across the pain scale, S, (where "0" = no pain,

"1" = slight pain,
"2" = moderate ",
"3" = severe pain,
"4" = very severe pain,
"5" = unbearable pain)

and so to write on the revolving smoked drum.

Decompression of bulb; B, by turning handle, H, in the reverse direction causes the pain-pointer to fall over the scale towards zero.

The pain-scale is so arranged on a large obscuring card that the subject cannot observe the configuration of the pain-picture which he makes.

Another scale, similarly operated is arranged to write above the pain-scale, in temporal alignment with it, and to record itch when this occurs. The itch scale is graduated as follows: -

"1" = moderate itch
"2" = severe itch.
Intradermal Injections in the same Subject.

(a) Acetylcholine - $10^{-4}$g/ml.
(b) 0.9% $\text{w/v}$ NaCl.

In each record:

i. = pain due to insertion of the needle.

ii. = painless period of duration unknown to the subject.

iii. = pain caused by the injection of fluid, 0.05 ml.

The Pain Scale used in this and in all subsequent figures is that described for Figure 1.

Subject:-

R.M.L.D.
Figure 3.

Pain Records.

**Intradermal Injections.**

One subject. Each strip shows the pain-response due to the insertion of the hypodermic needle, a blank period of duration unknown to the subject, and the pain-response elicited by one injection of 0.05 ml. test-fluid. The time of injection of test-fluid is indicated by an arrow in each case.

**Test solutions:**

NaCl, 0.9% w/v.

Acetylcholine $10^{-5} \text{g/ml.}$ and $10^{-2} \text{g/ml.}$

For meaning of (a) and (b) see text.
Figure 4.

Typical Cantharidin Blister on the Forearm, before Opening and Exposure of its Base.

Such a blister is produced by 0.3% cantharidin in a plaster of beeswax, wool fat and castor oil (1 cm. x 2 mm.) applied to the skin for 7 hr., 12 hr. before experiment.
Figure 5.

Pain Records.

Records made by two Subjects, (a) and (b) respectively, showing the typical Ability of Subjects to discriminate between various Concentrations of Acetylcholine applied to the Cantharidin Blister Area.

In record (a) an acetylcholine antagonist, atropine sulphate $10^{-4}g/ml.$, was applied as bathing fluid between the fourth and fifth test-substance administrations, instead of the usual R.L. (= Ringer-Locke) solution. Between the fifth and sixth (last) administrations R.L. was used as usual.

In record (b) plasma was used as a 'control' administration.

Concentrations are expressed as $g/ml.$ acetylcholine chloride and atropine sulphate.

Subjects:—

D.A.

J.W.M.
Figure 6.

Pain Records.

"Significant" Pain-responses to 5-hydroxytryptamine in low Concentration ($10^{-8}$g/ml. 5-HT creatinine sulphate).

Two subjects.

Subjects:

J.W.M.

R.M.L.D.
Figure 7.
Pain Record.

Record showing:

1. Maintenance of Sensitivity of the Pain-area and of Discrimination by the Subject during 1½ hours.


3. The Occurrence of slight to moderate Itch following Acetylcholine Pain-response.

Subject:

J.W.M.
Figure 8.

Pain Responses.

Duration of Refractoriness to Acetylcholine - the Effect of varying the Interval between Administrations on the Intensity of the Pain-response to Acetylcholine.

The (9) figure refers to the interval between the second application of a pair and the first application of the next pair.

Subject: -

R.M.L.D.
PAIN RESPONSES
TO ACH 5 x 10^{-4} gm/ml.

TIME-MIN

0 1 2 3 4 5 6 7 8 9

10 MIN.
Figure 9.

Pain Responses

The Effect of varying the Interval between Administrations on the Intensity and Duration of the Pain-response to KCl Solution.

Subject: -
C.A.K.
PAIN RESPONSES  (INTENSITY x DURATION)

TO KCl  7.7 mg/ml

TIME - MIN

PAIN

0  1  2  3  4  5  10

10'  2'  5'  10'
Figure 10.

Pain Record.

Progressive selective Depression of the Pain-area produced by 5-hydroxytryptamine (5-HT) and by Serum.

By the end of the experiment,(about 1½ hr.) pain stimulation by serum and by ten times the original concentration of 5-hydroxytryptamine was 'blocked' whereas the effect of the original concentration of acetylcholine was little affected.

Numbers above Fig. refer to concentrations of ACh in g/ml.

5-HT = 5-hydroxytryptamine creatinine sulphate in g/ml.

Latent Period. This refers to the period between application of solution to the blister area and the time of onset of pain-response to it. With acetylcholine the response is immediate and is not indicated. With serum and with 5-HT there is a delay which is inversely proportional to the concentration applied and to the sensitivity of the area.

Subject:

J.W.M.
Figure 11.

Pain Record.

Effect of varying the Rate of Administration of Acetylcholine to the Blister-area. The Necessity for rapid Drug Administration.

During the first part of the experiment, (a), the acetylcholine was rapidly applied and the subject distinguished between two concentrations of the drug, viz. $10^{-4}$ and $10^{-3}$g/ml. In the second part, (b), one concentration only, of acetylcholine was employed, the higher ($10^{-3}$g/ml), and its rate of administration was varied. When the acetylcholine was slowly administered the pain responses to it were poor, not exceeding those to 1/10 the concentration rapidly applied (see (a), $10^{-4}$g/ml. ACh).

subject: -

C.A.K.
The Effect of Blister-area Size on the Pain-response to Acetylcholine. Preference for the Use of Areas of Diameter greater than 0.5 cm.

The effective size of the blister-area was varied by the application of easily removable low-melting-point paraffin wax.

When the area was reduced the subject failed to distinguish between $5 \times 10^{-5}\text{g/ml.}$ and $10^{-3}\text{g/ml.}$ acetylcholine. On the total area not only could he distinguish between $5 \times 10^{-5}\text{g/ml.}$ and $10^{-3}\text{g/ml.}$ acetylcholine but also between $10^{-5}\text{g/ml.}$ and $5 \times 10^{-5}\text{g/ml.}$ On the whole area the intensity of pain elicited by a given concentration is greater than that obtained on the small area. The figure shows that $10^{-5}\text{g/ml.}$ on the whole area elicited as much pain as did $10^{-3}\text{g/ml.}$ applied to the reduced area.

Subject: -

C.A.K.

a. (i) shows the immediate pain response to acetylcholine administration, and the subsequent rapid decline of the pain. (ii) shows the slightly delayed (2 sec), though longer lasting, response to a KCl administration which produced pain of a similar order of intensity to that produced by ACh in (a) (i).

b. Here a third substance, 5-HT (administration (ii)), producing an even greater intensity of pain than the immediately acting ACh (response (i)) does so only after a prolonged latent period (19 sec). The response is then long maintained.

Subjects:

C.A.K.
R.M.L.D.
The figure shows the 'atypical' pain responses to acetylcholine administered as the first application of an experiment within \( \frac{1}{4} \) hour of opening the blister. Two subjects (a) and (b) respectively. The unusual responses are accompanied by 'low' sensitivity of the area to acetylcholine at the time of the first administration as compared with that at the times of subsequent application.

Subjects—

W.J.W.

C.A.K.
Figure 15.

Pain Record.

Variations in Pain-pictures. Filming Over.

The first part of the record shows that
5 x 10^{-5} g/ml ACh produced no pain and 10^{-4} g/ml.
very little (-almost \frac{1}{2} unit intensity). It
was then noticed that a film had formed on the
area. 'At X' the area was rubbed to remove the
film. 5 x 10^{-5} g/ml. ACh then produced severe
pain (3\frac{1}{2} units intensity) and even 10^{-5} g/ml. was
quite definitely painful (1 unit intensity).
This increased sensitivity was then maintained
throughout the experiment.

Subject:—

C.A.K.
Figure 16.

Pain Responses.

Effect of prior Administration of Acetylcholine on the Pain induced by KCl Solution applied to the Blister-area.

The diagram shows pain-responses to two concentrations of ACh solution. The responses are proportional to the concentrations applied. It also shows two responses to a single concentration of KCl. With the exception of the final administration the time-interval between doses is ten minutes. The second KCl administration the final of the series, was made immediately (5 sec) after that of the high concentration of ACh. Little percentage lowering of the KCl response occurred. (Compare Fig. 17).

Subject:—

C.A.K.
PAIN
INTENSITY x DURATION

\[ \text{KCl} 0.77\% \]
\[ \text{ACh} 3 \times 10^{-3} \text{g/ml} \]
\[ \text{ACh} 10^{-2} \text{g/ml} \]
Effect of prior Administration of KCl Solution on the Pain induced by Acetylcholine applied to the Blister-area.

The diagram shows pain-responses to two concentrations each of ACh and of KCl solutions applied to the area at ten minute intervals. The responses are proportional to the concentrations used. When, however, the acetylcholine is administered immediately (5 sec) after the response to KCl the ACh response is very greatly diminished.

Subject:-

C.A.K.
PAIN INTENSITY x DURATION

\[ ACh \ 10^4 g/ml. \]
\[ ACh \ 5 \times 10^{-4} g/ml. \]
\[ KCl \ 0.38\% \]
\[ KCl \ 0.77\% \]
Figure 18.

Pain Records.

Actions of Cantharidin Blister Fluid (a), and Burn Blister Fluid, (b) on the Cantharidin Blister Area.

This shows that the effect of fluid from a Cantharidin blister is not due to the Cantharidin in the fluid.

(For meaning of a' and b' see a and b, Fig. 53).

Subjects:

J.W.M.
D.E.A.
Figure 19.

Pain Records.

Actions of:
- Ringer-Locke Solution,
- 5-hydroxytryptamine and
- Acetylcholine

on the Bases of Blisters induced by
- Cantharidin - (a)
- Heat - (b)

This record shows that the sensitivity of the blister base is not influenced by cantharidin.

Subjects:
- C.A.K.
- J.W.M.
Figure 20.

Pain Records.

Pain Responses to
(a) HCl,
(b) HCl and Lactic Acid,
(c) Citric Acid,

applied at various pH's.

It can be seen that pH's below 3 regularly produce pain whereas those above 3.5 do not.

Subjects:

C.A.K.
D.A.
C.A.K.
Figure 21.

Pain Records.

Pain-producing Actions of distilled water, (a), and 5% NaCl, (b).

Subjects:

J.W.M.

R.W.H.E.
Graph showing the Effects of Cocaine hydrochloride $2 \times 10^{-4}$, (O---O), and $10^{-3}$, (●---●), g/ml. on the Pain-response to Acetylcholine chloride, $6 \times 10^{-5}$g/ml. when the former was applied to the Blister-area for 10 min as Bathing Fluid.

Subject:—

J.W.M.
PAIN

MINUTES
AFTER REMOVAL
OF COCAINE
Figure 23.

Pain.

Graph showing the Effects of Nicotine tartrate $10^{-4}$, (O------O) and $5 \times 10^{-4}$, (●—●) g/ml. on the Pain-response induced by Acetylcholine chloride $6 \times 10^{-5}$g/ml. when the former was applied for 10 min as Bathing Fluid.

The figure indicates the original pain-response to ACh (1½ units) then the responses at five minute intervals following the removal of the nicotine solution.

Subject:

J.W.M.
PAIN

MINUTES
AFTER REMOVAL
OF NICOTINE
Carbachol

Carbachol is much less pain-producing than ACh — here of the order of one tenth or less.

Subject:—

B.C.
Figure 25.

Pain Record.

Carbachol - second subject.

Here the potency of the carbachol is shown to be one twenty fifth, or less, than that of the ACh.

Subject:—

D.A.
Figure 26.

Pain Records.

Blister-area Responses to:

(1) Acetylcholine chloride \( \text{ACH} \)
(2) Methacholine chloride \( 'M' \)
(3) Pilocarpine nitrate \( 'P' \)
(4) Nicotine tartrate \( 'Nicotine' \)
(5) Sodium tartrate \( 'NaT' \).

Subject:

C.A.K.
Antagonism of ACh Responses by

d-tubocurarine.

Between administrations 1 and 5 the area
was bathed in R.L. (= Ringer Locke solution) in
the usual manner (not indicated). Between
applications 5 and 6 of ACh the bathing fluid
contained d-tubocurarine chloride (d-to) $10^{-5} \text{g/ml.}$
(indicated: $\overset{\downarrow}{\text{d-to } 10^{-5}}$). The tubocurarine is
shown to reduce the pain of application of $10^{-4}$
$\text{g/ml. ACh (over 2 units, on scale) to zero. The}$
subsequent use of R.L. as bathing-fluid almost
completely restored the activity.

Subject:-

R.M.I.D.
Figure 28.

Pain Record.

Antagonism of ACh-induced Pain by

Hexamethonium and by

Decamethonium.

Between applications 2 and 3 of ACh the bathing-fluid contained decamethonium iodide $10^{-5} \text{g/ml}$. and the pain-response to $10^{-4} \text{g/ml}$. ACh was almost completely abolished.

Between applications 4 and 5 hexamethonium bromide $10^{-4} \text{g/ml}$. was applied and this depressed the pain-response to $10^{-4} \text{g/ml}$. ACh only slightly.

The last part of the tracing shows the prolonged depressant action of $10^{-3} \text{g/ml}$. decamethonium on ACh-induced pain.

Subject:

C.A.K.
Figure 29.

Pain Record.

Hexamethonium

Hexamethonium bromide $10^{-4}$ g/ml. applied to the blister-area did not depress the response to $10^{-4}$ g/ml. acetylcholine, though $10^{-3}$ g/ml. did.

Subject: -

J.W.M.
Figure 30

Pain Records.

**Procaine**  
**Cinchocaine**

(a) shows the effect of procaine hydrochloride $10^{-4} \text{g/ml.}$ applied as a bathing fluid between ACh applications 1 and 2. The procaine was removed by blotting before application 2. It was replaced for 3 min before application 3 and for a further 5 min before application 4. After this the procaine was well washed off and R.L. used once again as the bathing fluid. After 20 min the sensitivity of the area to ACh was almost completely restored.

(b). This record shows the action of cinchocaine hydrochloride $10^{-5} \text{g/ml.}$ applied between responses 1 and 2, and of $10^{-4} \text{g/ml.}$ applied between administrations 2 and 3 of acetylcholine.

Applications 4, 5 and 6 were made in rapid succession to see which concentration of ACh could 'break through' the cinchocaine blockade.

The area was then bathed in R.L. solution for ten minutes at which time the area was still unresponsive to both $5 \times 10^{-5}$ and $10^{-3} \text{g/ml.}$ ACh.

After a further 10 minutes the sensitivity of the area was almost completely restored.

Subject: - J.L.M.
Figure 31.

Pain Record.

Neostigmine

Between ACh applications 4 and 5, and 5 and 6 neostigmine sulphate $10^{-4}$g/ml. was applied as bathing-fluid. There was no increase in the response to the 'threshold' ACh administration.

Subject:-

J.W.M.
Figure 32.

Pain records.

$K^+$, administered as KCl Solution.

(a) shows the different time courses of pain responses to concentrations of ACh and KCl which produce the same intensity of pain.

(b) illustrates these differences in time-course which occur with concentrations of KCl producing 'slight' pain (response 2) and those producing 'moderate' to 'severe' pain (response 3).

This shows that just as the characteristic ACh responses are properties of the substance rather than of its concentration so, with KCl, the usually delayed onset of response, the slow climb to peak, and the prolonged duration in action are properties of that material rather than of its concentration.

Subjects:—

J.W.M.
C.A.K.
Figure 33.

Pain Records.

**Adrenaline; Nor-adrenaline; Ephedrine.**

(a) Adrenaline, administered as tartrate g/ml. - 'A'
    Sodium tartrate, g/ml. - 'NaT'

(b) Nor-Adrenaline, administered as tartrate,
    g/ml. - 'N'

(c) Ephedrine, administered as hydrochloride,
    g/ml. - 'E'

Subjects:

Chapman
Figure 34.

Pain Records.

Histamine.

The Figure shows the production of itch only by histamine dihydrochloride $= 10^{-5}\, \text{g/ml.}$ base, and of pain and itching by $= 10^{-3}\, \text{g/ml.}$ base.

The histamine pain is early in onset (less than 15 sec delay) whereas the induced itch occurs at about one minute.

Subject:

C.A.K.
Figure 35.

Pain Records

Effect of Histamine on Acetylcholine-induced Pain of threshold Intensity.

Record (a) shows no potentiation of the pain induced by $10^{-5}\text{g/ml. ACh}$ when histamine dihydrochloride $= 10^{-4}\text{g/ml.}$ base was administered with it. The apparent potentiation when $= 10^{-3}\text{g/ml.}$ histamine base was used in the mixture, was due to the action of the histamine itself since record (b) shows that this concentration of histamine could produce almost as much pain.

Subject:--

R.M.I.D.
Figure 36.

Pain Responses

Compound 48/80 (Paton, 1951)

This figure shows pain and itch following the application of histamine-liberating compound 48/80, $5 \times 10^{-4} \text{g/ml}$, to the blister base. The pain occurred after about half a minute's delay and the itch followed a minute after that.

Subject: --

J.W.M.
Tryptamine; Tryptophan.

The figure shows that though tryptamine hydrochloride $10^{-5}$ g/ml. is pain-producing tryptophane $10^{-3}$ g/ml. is not.

(The molar weights of these substances are almost identical).

Subject:--

R.M.L.D.
1 min.

Ach. 5 x 10^-5 g/ml
Trypto-Phane 10^-3 g/ml
Trypt-AMINE 10^-5 g/ml
Figure 38.

Pain Record

Tryptamine.

Delayed, long-lasting moderate-to-severe pain induced by tryptamine hydrochloride $10^{-6}$ g/ml.

Subject:-

R.M.L.D.
5-Hydroxytryptamine.

(a) Severe pain induced by $10^{-7} \text{g/ml.}$
5-hydroxytryptamine creatinine sulphate.

(b) Pain production by $10^{-8} \text{g/ml.}$
5-hydroxytryptamine creatinine sulphate
($\equiv 5 \times 10^{-9} \text{g/ml. 5-HT base}$). This compound is
shown to be at least ten times as active as
tryptamine hydrochloride ('T';g/ml.) (The
molar weights of these substances are in ratio
2/1 which indicates that the 5-OH compound is
in fact at least twenty times as potent as the
other, on a molar basis).

Subject:-

J.W.M.
Figure 40.
Pain Record.

Tryptamine and $\alpha\alpha$ dimethyl tryptamine

This record indicates that tryptamine is between 10 and 100 times more active in producing pain than the $\alpha\alpha$ dimethyl substituted compound.

The substances were administered as dimethyl tryptamine acetate and tryptamine hydrochloride and the concentrations indicated are molar.

Subject:

J.B.J.
DIMETHYL TRYPTAMINE and TRYPTAMINE
Figure 41.

Pain Records.

(a). 5-hydroxytryptamine and 5-hydroxy NN dimethyl tryptamine.

(b). NN dimethyl tryptamine and 5-hydroxy NN dimethyl tryptamine.

Record (a) shows reduction in potency by introduction of NN dimethyl substitution in the 5-HT side chain. \( B = 5 \)-hydroxy NN dimethyl tryptamine, bufotenin. The potency is less than one tenth that of the unsubstituted compound.

Record (b) shows pain-production by 5-hydroxy NN dimethyl tryptamine, bufotenin, \( B \), and lack of stimulation by equivalent concentration of the compound lacking the 5-OH group, viz. NN dimethyl tryptamine, NNDT.

The following salts were used:

5-HT — as creatinine sulphate.

Bufotenin — acetate.

NN dimethyl tryptamine — acetate.

The concentrations are expressed as molar.

M.

Subject: —

D.A.
Figure 42.

Pain Record.

Bufotenin.

Pain production by $10^{-6}\text{g/ml.}$ bufotenin, 5-hydroxy NN dimethyl tryptamine. The pain is delayed in onset, as for 5-HT induced pain, slow-mounting and long-lasting.

Subject:—

Read
Figure 43.
Pain Record.

4- and 5- hydroxy tryptamines.

The record shows that 4-hydroxy tryptamine is less pain-producing than the 5-hydroxy compound.

The compounds were administered as creatinine sulphates.

Subject:—

J.B.J.
4- and 5-HYDROXY TRYPTAMINES

PAIN

4-HT
5x10^{-7}_M

5-HT
5x10^{-6}_M

4-HT
5x10^{-5}_M

5-HT
5x10^{-5}_M

23°

15°

8°

11°
Figure 44.

Pain Record.

5- and 6- hydroxy tryptamines.

This shows that 5-hydroxytryptamine is also more potent than the 6-hydroxy compound, having between ten and one hundred times the pain-producing capacity of the latter.

The compounds were administered as the creatinine sulphates.

Subject:—

J.B.J.
5- and 6-HYDROXY TRYPTAMINES

ACh
5x10^4 M
10'

6-HT
5x10^-7 M
20''

5-HT
5x10^-7 M
19''

6HT
5x10^-6 M
5''
Figure 45.
Pain Record.

Gramine.

Absence of pain-production by indolyl methyl dimethyl amine, gramine. (Molar weights of the compounds compared, gramine and 5-hydroxytryptamine creatinine sulphate, are in ratio of 1/2+)

Subject:—
Read.
Figure 46.

Pain Records.

Pain Production by Serum (a) and by 5-hydroxytryptamine (b).

Notice the long latent period before onset of pain both with serum (here, 40 sec) and with the 5-HT creatinine sulphate, '5-HT', $10^{-6}$ g/ml. (here, 19 sec), also the slow build up and prolonged maintenance of the pain in both cases.

Subject:

R.M.L.D.
(a) ACh, Serum
10^{-4}

(b) ACh, 5-HT
10^{-4}, 10^{-6}
Figure 47.

Pain Record.

Adenosine triphosphate.

Adenosine triphosphate is shown to be much less pain producing than 5-hydroxytryptamine.

Adenosine triphosphate administered as the disodium salt,
5-hydroxytryptamine administered as the creatinine sulphate.

(Ratio of the molar weights is approximately 1.5/1 thus making the 5-HT about sixty times more potent than the ATP).

Subject:-

D.E.A.
Figure 48:

Pain Records.

Posterior Root Extract.

The figure shows pain production by the reconstituted (in saline) dried acetone extract of the posterior root nerves of the horse. (see Holton and Holton, 1952). In terms of parent dorsal root, wet weight, the doses used, per ml. solution, correspond to five times those used of the dried powdered extract. For comment see text.

Subject: -

J.W.M.
Figure 49.

Pain Records.

Angiotonin; Bradykinin; Substance P.

This figure shows that angiotonin, bradykinin and substance P are all pain-producing and in all cases the pain is delayed in onset, slow-mounting and of prolonged duration.

Subject:

C.A.K.
Vasopressin; Leukotaxine.

(a) Vasopressin – Pitressin (Roche). In a concentration of 1 unit/ml. this produced typical polypeptide delayed prolonged pain.

(b) A leukotaxine preparation in concentration of $10^{-4}$ g/ml. behaved similarly.

Subject:—

C.A.K.
Figure 51.

Pain Records.

Pain Production by A, Fluid from an Insect bite Blister.
B, Fluid from an arthritic Joint.
C, A pleural Effusion of infective Origin

These fluids, all of human origin, were collected in a glass syringe and applied to the blister area a few minutes later. In all cases the pain was delayed, slow-mounting and prolonged. If, however, the fluid were kept at room temperature for an hour or more it ceased to be pain-producing.

A (a) shows lack of pain produced by insect bite fluid kept at room temperature for 3½ hr.
(b) shows the effect of the same fluid deep frozen (-20°C) immediately after collection and then brought to room temperature for five minutes before its application.

Subjects:
(a) Phillips
(b) and (c) C.A.K.
Figure 52.

Pain Records.

Cantharidin Blister Fluid - (a) 'Fresh'

(b) 'Old.'

(a). This fluid had been kept in glass at room temperature for 25 min. only.

(b). Shows absence of effect by the same fluid kept at room temperature for three hours.

Subject:

J.W.M.
Figure 53.

Pain Record.

Burn Blister Fluid (a) 'Old'

(b) 'From the Deep Freeze'

(a) This fluid had been kept in glass at room temperature for 1½ hr.

(b) This aliquot of the same fluid had been placed in the deep freeze (−20°C), immediately after collection and applied to the blister-area five minutes after unfreezing. There was delay of 25 sec between application of the fluid and the onset of pain which built up to very severe intensity and lasted during several minutes.

Subject:

D.E.A.
Figure 54.
Pain Record.

Serum.

The effect of application of a subject's own serum to his blister area. The delayed, slow-mounting prolonged pain resembles that induced by tryptamines, certain pathological effusions and by certain polypeptide preparations.

Subject:

R.M.L.D.
Figure 55.
Pain Record.

Plasma and Serum (− age 2 hr).

The subject responds to the serum but not to the plasma.

Subject:

R.M.L.D.
Figure 56.

Pain Record.

Dialysate of Human Serum.

When human serum is dialysed in cellophane against R.L. solution during 24 hr. an agent with the same pain-producing property passes out into the R.L. solution. The molecule of active principle is therefore apparently not large and is independent of protein.

Subject:—

R.M.L.D.
Figure 57.

Pain Record.

Saline Extract of broken human Platelets.

'Control' = extract from plasma rendered platelet-poor.

'Platelet Extract' = extract from the same parent plasma having been rendered platelet-rich.

The pain produced resembled that due to serum.

Subject:

R.M.L.D.
Figure 58.

Pain Records.

Pain Production by (a) Heparinised human Plasma. (b) Citrated human Plasma.

The plasmas had been kept in glass at R.T. during half an hour.

(a) and (b) show that the effect is not related to the particular anticoagulant used. (b) shows also absence of effect from $10^{-2}$g/ml. sodium citrate and from citrated blood of total 'age' one min.

Subject:

Mestitz.
Figure 59.

Pain Record.

Heated human Plasma.

This figure shows the effect on human plasma of heating it in a polythene container immersed in a 70°C water-bath and of the same plasma kept similarly but at 37°C. The heated sample was pain-producing and the pain itself was delayed and prolonged.

Subject:—

C.A.K.
PAIN

HUMAN PLASMA

37° 70°

20min 2min
Figure 60.

Pain Records.

Extracts of (a) Rat, and
(b) Human, skin in
Ringer-Locke Solution.

The curved records shown in (a) were made by a side-writing lever.

Skin-extract pain is delayed. It usually induces itching also.

Subjects:—

J.W.M.

D.E.A.
a. ACh RAT 10^-4 SKIN EXTRACT

b. ACh 10^-5 ACh 5x10^-5 HUMAN SKIN EXTRACT
Figure 61.

Rat Uterus Contractions.

Responses of the isolated Rat Uterus to:

Cantharidin Blister Fluid - 'C.B.F.'
Burn Blister Fluid - 'B.B.F.'
Insect bite Blister Fluid - 'I.B.F.'
Hydrocoele Fluid - 'Hc.F.'
Ascitic Fluid of infective Origin - 'Asc.F.'
Joint Fluid - arthritic - 'J.F.'
Rat Skin Extract - 'R.Sk.E.'
Bradykinin - 'Bk'
Human plasma - 'H.Plasma.'

The records illustrate contractions, after a latent period, in response to the above fluids.
Figure 62.

Rat Uterus Contractions.

Responses to: KCl
ACh
Histamine
5-HT and
Bradykinin.

The figure illustrates the marked insensitivity of the isolated rat uterus to K$^+$ and to histamine alongside normal sensitivity to low concentrations of ACh, 5-HT and bradykinin.

Doses are measured in g.

KCl $1.15 \times 10^{-2}$g/ml. = isotonic
H = histamine dihydrochloride
5-HT = 5-hydroxytryptamine creatinine sulphate;
Bk = bovine bradykinin preparation.
Figure 63.
Rat Uterus Contractions.

Rat Blood.

'Alc. Ext. Human' = alcoholic extract, dried and reconstituted, of glass-activated human plasma.

It was used to indicate the sensitivity of the tissue.

The rat's blood was collected into a siliconed syringe. The applications at t = 0 and "o" refer to administrations from this syringe. Some of this blood was transferred to a glass vessel. Applications at 3', 10', 18', and 25' refer to the durations of exposure of this blood to glass.

The responses to the rat blood, when these occur, resemble those to the human plasma and inflammatory exudates shown in Fig. 61.
Alc. Ext.  Rat Blood
Human  t = 0  3'  10' "0"  18'  25'
0.05  mil  0.05  0.05  0.05  0.5  0.25  0.25
ml  ml  ml  ml  ml  ml  ml
Figure 64.

Rat Uterus Contractions.

Decay of Activity - Burn Blister Fluid
- Insect-bite Blister Fluid.

(a) Burn blister Fluid. This fluid was collected into a glass syringe and deep-frozen. On removal from the cold, and melting, it was found to be highly oxytocic (t = 0). Administrations were then made at ten minute intervals up to 50 min. The final administration, 't = 0' was made from another aliquot of the same fluid freshly unfrozen as was done for administration t = 0. This fluid from the deep freeze was highly active and indicated that the declining contractions of the uterus following constant volume administration were due to decay of active principle at R.T. and not to diminished uterine responsiveness.

(b) Insect bite blister fluid. This tracing was made in the same way as for the (a) record.
Figure 65.
Rat Uterus Contractions.

Decay of Activity - Joint Fluid (rheumatoid arthritic) - (a).
- Pleural Effusion of infective Origin - (b)

These records were made in analogous manner to those in Fig. 64.

(a) **Joint fluid.** By 24 min after removal from the deep freeze the fluid was no longer oxytocic though five times the previous dose could elicit a response \( t = 32 \) min. After a further 8 min this activity also had disappeared \( t = 40 \) min though the sensitivity of the tissue to the original dose (0.07 ml.) of fluid 'straight' from the deep-freeze was almost unimpaired.

(b) **Pleural Effusion.** Responses of the uterus to various doses of this effusion at various times after removal from the deep freeze, where it was stored after collection in a glass syringe, are shown.
(a) JOINT FLUID 30 ml. bath

3 min

JOINT

66 ml.

16 min 24 min 32 min 40 min 't = 0`

0.07 ml.

(23 μl/ml)

(b) PLEURAL EFFUSION 30 ml. bath

3 min

PLEURAL EFFUSION

30 min 28 min 35 min 54 min

0.05 ml.

(2 μl/ml)

20 min

0.15 ml.

(6 μl/ml)

(8 μl/ml)

0.25 ml.

(16 μl/ml)
Figure 66.
Rat Uterus Contractions.

Decay of Activity  -  Human Plasma.

Human blood was collected into heparin and spun in glass to 'make' plasma which was then stored some in the deep freeze, and some at 0°C till time of experiment.

Experiment. Some of the deep frozen material was brought to room temperature and immediately tested - D.F.; some of this unfrozen plasma was allowed to remain at room temperature and was then tested at various time intervals - R.T. 3 min, 15 min etc.; 0°C indicates the plasma which was kept at that temperature after the centrifuging.

It will be seen that the samples D.F. (from the deep freeze) are active with comparable 'heights' of contraction and similar latent periods whereas those samples kept at R.T. induce smaller responses which occur after progressively longer latent periods.
Figure 67.
Pain Record.

Cardiac Oedema Fluid.

Absence of response to cardiac oedema fluid whether tested straight from the siliconed collection apparatus (- "Pre-active C.O. Fluid") or after exposure to glass for 5 or 10 min. ("C.O. Fluid G 5"; "C.O. Fluid G 10").

This fluid was of low protein content (0.6%).

Subject: -

Pearce
Onset of Activity - Cantharidin Blister Fluid.

(a) Pain. The subject had two cantharidin blisters - one to provide the blister area and the other to supply absolutely fresh blister fluid when required.

After the experimental area had been exposed and had 'settled down' ready for experiment the fluid was aspirated from the second blister to a glass syringe and applied immediately to the test area and to

(Subject: - J.B.J.)

(b) the rat's uterus in rapid succession.

Neither tissue responded

When, however, the blister fluid was applied to the rat uterus after 6 min in the syringe and to the pain area at 20 min responses were obtained from both tissues.
PAIN

(a)

PAIN

ACh
10^{-4}
g/ml.

C.B.F.
at 20 sec

C.B.F.
at 20 min

RAT UTERUS

30 ml. bath

(b)

5-HT
5 \times 10^{-8}
a/ml.

5-HT
2 \times 10^{-7}
a/ml.

C.B.F.
at 20 sec
0.1 ml.

C.B.F.
at 6 min
0.05 ml.
(a) Pain. The subject's own blood applied to his blister area at a total age of 2 min after inserting the collecting needle into his vein produced no pain. His heparinised plasma, total age 7 min, produced delayed prolonged pain.

Subject: -

D.E.A.

(b) Uterus - actions on this tissue of heparinised blood and plasma of the same age, viz. 2 and 7 min. The effects can be seen to run in parallel with the pain-area responses.
Figure 70.

Human Pain: Rat Uterus Contractions.

Onset of Activity. Activation of 'Pre-active' Human Plasma ('P.a.p.') by Exposure to Glass.

Top record - pain. (Subject: - C.A.K.)
Lower record - uterus

It can be seen that neither preparation responded to the plasma which had never been in contact with glass, (p.a.p.). Exposure of the plasma to glass for 7 min (G 7) produced a pain and uterine stimulating material.

Pain - note its delayed onset and prolonged type. Application for 1 min only.

Uterus - note that the volume of glass-activated plasma which induced the pronounced early contraction was half that which produced almost completely negative effect. Tissue exposure 1 min.
Figure 71.
Human Pain: Rat Uterus Contractions.

Ethyl Alcohol Extracts of 'pre-active',
glass activated and
decayed Human Plasma

The extracts were made in boiling ethyl alcohol from plasma which had been exposed to glass for 40 min (- 'Decayed'), not at all (- pre-active plasma, 'p.a.p.') and 7 min (- 'g.a.p.').

Alo. Ext. = alcoholic extract.

(a) Pain. (Subject:- C.A.K.)
(b) Uterus.

On both tissues the 'decayed' has little effect, the pre-active no effect, and the glass activated pronounced stimulating activity.

(Note - On the rat uterus the stimulating solution was applied in one quarter the dose of the inactive fluids).
Figure 72.

Human Pain: Rat Uterus Contractions.

Heated Human Plasma.

Top record - pain. (Subject C.A.K.)
Lower record - uterus.

It can be seen that the plasma heated in the 69°C bath produced pain on application to the blister base and also stimulated the rat uterus whereas that kept at 37°C stimulated neither tissue.

(Note the dose of stimulating solution to the uterus is one half that of the fluid which induced no response).

A.E. = alcoholic extract of glass
activated human plasma.
PAIN

Ach $10^{-4}$  
Plasma 2 min in bath $37^\circ$  
Plasma 2 min in bath $69^\circ$

RAT UTERUS

5 ml. bath

AE 0.05 ml.
Plasma 0.1 ml.
Plasma 0.05 ml.

$37^\circ$  
$69^\circ$  
$100^\circ$  
$10^\circ$
Figure 73.  
Isolated Rabbit Gut.  

Cantharidin Blister Fluid.  

The record shows the action (inhibition of activity followed by increase in tone and restoration of rhythmicity, gradually wearing off) of cantharidin blister fluid applied "to the tissue," at 6 min after collection to a glass syringe.
RABBIT GUT

30 ml. bath

C.B.F. 0.05ml at 6 min
Figure 74.

Isolated Rabbit Gut.

Decay of Activity - Cantharidin Blister Fluid.

The lower record was made 2 hr. after the upper. Wash-kicks are shown throughout.

The responses of the tissue to acetylcholine are constant and to R.I. are absent.

The upper record shows the typical action of cantharidin blister fluid (C.B.F.) when freshly collected to a glass syringe (here 3 min old). The lower record shows the absence of effect of the same fluid when old (2 hr).

Subject:

J.W.M.
Activation and Decay of Human Plasma.

Top record - guinea-pig ileum (M. Rocha e Silva)
Lower record - rat uterus.

Bk = bovine bradykinin
P.a.p. = 'pre-active' human plasma
G3, G7, etc. indicates duration of the subsequent exposure of this plasma to glass.

It will be seen that the plasma shows parallel activities on both tissues.
Figure 76.
Guinea-pig Ileum: Rat Uterus.

Activation of Rat Plasma: non-activation of Rabbit Plasma.

(a) Guinea-pig ileum (– M. Rocha e Silva).
(b) Rat uterus

Bk = bovine bradykinin
P.a.p. = 'pre-active' plasma.
G3, G7 etc. indicates durations of exposure of plasma to glass.

The actions of the plasmas on the two tissues run in parallel.
GUINEA PIG ILEUM
5 ml. bath

RAT
0 0 0 5
Bk 20 0 25 5 0 25 
μg ml. μg ml.

RABBIT
15 0 25 0 25

(a)

RAT
Bk Pap Bk G3 Bk G3 Pap G3
0 5 0 0 5 2 1 0 0 5 0 0 5 0 1 0 1 0 0 1 0 0 2 0 4
μg ml. μg ml. ml. ml. μg μg μg

RABBIT

(b)

RAT UTERUS 30 ml. bath
Figure 77.

Guinea-pig Ileum: Rat Uterus.

Decay of Rat Plasma Activity.

(a) Guinea-pig ileum (M. Rocha e Silva).
(b) Rat Uterus.

Rat plasma was made by the silicone polythene technique and then exposed to glass.
G3, G4 etc. indicates the duration of exposure of the plasma to glass.
Bk = bovine bradykinin.
Parallel activity is seen on both tissues.
Figure 78.
Guinea-pig Ileum: Rat Uterus.

Bradykinin.

Top record — guinea-pig ileum (- M. Rocha e Silva)
Lower record — rat uterus.

Responses to bovine bradykinin.
For discussion see text.
BRADYKININ — µg
(BOVINE)

GUINEA PIG
ILEUM

5 ml. bath

RAT
UTERUS

30 ml. bath
Figure 79.

Rat Uterus Contractions.

Action of Atropine on Contractions induced by Acetylcholine and by Burn Blister Fluid.

Atropine sulphate – $10^{-6} \text{g/ml. final concentration in the bath was used.}$

The acetylcholine equivalent of the burn blister fluid breaking through the atropine blockade would be $50 \text{mg/ml. B.B.F.}$

B.B.F. = burn blister fluid.
RAT UTERUS
30ml BATH

2min

ACH ACH ACH ACH ACH ACH ACH BBF ACH ACH
$4 \times 10^{-7}$ $2.5 \times 10^{-7}$ $3.5 \times 10^{-7}$ $3.5 \times 10^{-7}$ $3.5 \times 10^{-7}$ $3.5 \times 10^{-4}$ $10^{-3}$ $0.02$ $10^{-3}$ $10^{-3}$

ATROPINE $10^{-6}$ g/ml.
Figure 30.

Rat Uterus Contractions.

Responses to:

ATP  =  Adenosine Triphosphate, disodium salt.

5-HT  =  5-hydroxytryptamine creatinine sulphate.

B.B.F.  =  burn blister fluid

I.B.F.  =  insect bite blister fluid

The A.T.P. equivalents of these exudates would be several mg/ml. fluid. The dose response relationships of ATP and the blister fluids are dissimilar.
<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>5-HT</th>
<th>BBF</th>
<th>5HT</th>
<th>I.B.F</th>
<th>I.B.F</th>
<th>I.B.F</th>
<th>ATP</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>3min</td>
<td></td>
<td>1 x 10^7</td>
<td>4 x 10^7</td>
<td>0.1</td>
<td>3 x 10^7</td>
<td>0.1</td>
<td>0.03</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>g</td>
<td>ml.</td>
<td>g</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
</tr>
</tbody>
</table>

RAT UTERUS
30 ml BATH
Figure 81.
Rat Uterus Contractions.

Stability of 5-HT added to Inflammatory Exudate.

5-HT = 5-hydroxytryptamine creatinine sulphate, g.
Inflammatory exudate = burn blister fluid, B.B.F.

Two aliquots of the same burn blister fluid were taken and 5-HT was added to one in the concentration of $3 \times 10^{-6}$ g/ml. Both were left at R.T. The one containing only B.B.F. was tested on the uterus at 10 min intervals indicated by $t = 0$, 10 min, 20 min, etc. The activity of 0.1 ml. had disappeared at 20 min. Ten minutes later the aliquot containing the 5-HT was tested in a dose to give 0.1 ml. B.B.F. and $3 \times 10^{-7}$ g 5-HT (Mixture = M). The response was as for $3 \times 10^{-7}$ g 5-HT. Ten minutes later, at 40 min, 0.2 ml. B.B.F. gave no response. At 50 min, however, 2M gave a response equivalent to more than $5 \times 10^{-7}$ g 5-HT.
Rat Uterus Contractions.

Acetone Precipitate and Extract of Burn Blister Fluid.

5-HT = 5-hydroxytryptamine creatinine sulphate.
Ext. = extract.
Ppt. = precipitate.

The extraction was made in 95% acetone in the cold (0°C). The volumes of 'extract' and 'precipitate' administered are equivalent to the volume of parent blister fluid from which the material was derived.
Figure 82(b)

Rat Uterus Contractions.

Acetone Precipitate of Burn Blister Fluid — Decay of Activity.

\[ P = \text{reconstituted precipitate.} \]
\[ 5\text{-HT} = 5\text{-hydroxytryptamine creatinine sulphate, g.} \]

The precipitate of Fig. 82(a) was administered at various time intervals after dissolving in R.I. solution (\( t = 1 \text{ min,} \)
25 min, 33 min). The final administration of \( P \) at '2 min' is of solution which had been kept in the cold during the experimental period and is seen to have maintained its activity.
Figure 83.
Rat Uterus Contractions.

Acetone Extract of Burn Blister Fluid to which 5-HT had been added.

5-HT = 5-hydroxytryptamine creatinine sulphate.
E = acetone extract.
3X, 12X, etc. indicates $3 \times 10^{-8} \text{g}$, $12 \times 10^{-8} \text{g}$.

E = 12X, means acetone extract of burn blister fluid containing 5-HT and the dose given was that equivalent to $12 \times 10^{-8} \text{g} 5HT$ of the mixture before extraction.

(a). The activity of the dose of extract is equivalent, in terms of 5-HT to over $10 \times 10^{-8} \text{g}$.

(b). The activity of the extract is stable during the 2 hr experimental period.
RAT UTERUS — 30 ml. bath.

(a) 5-HT 5-HT 5-HT 5-HT E = 5-HT
5x10⁻⁸ 3x 12x 5x 10x 12x 15x

(b) contd. E = 5-HT 5-HT 5-HT
12x10⁻⁸ 15x 12x 12x
9 2hr
Figure 84.

Rat Uterus Contractions.

Antagonism by Dihydroergotamine of the Actions of 5-HT on the Uterus but NOT those of
    Burn Blister Fluid,
    Joint Fluid, or
    Insect-bite Fluid

5-HT = 5-hydroxytryptamine creatinine sulphate.
B.B.F. = burn blister fluid.
J.F. = joint fluid from an arthritic joint.
I.B.F. = insect-bite blister fluid.
D.H.E. = dihydroergotamine; the concentration
given is the final concentration in
the bath.
Figure 85.
Rat Uterus Contractions.

Antagonism by Lysergic acid diethyl amide of the Actions of 5-HT on the Uterus but NOT those of Burn Blister Fluid or of Joint Fluid.

5-HT = 5-hydroxytryptamine creatinine sulphate
B.B.F. = burn blister fluid.
J.F. = joint fluid from an arthritic joint.
L.S.D. = lysergic acid diethyl amide; the concentration given is the final concentration in the bath.
Figure 86.
Rat Uterus Contractions.

Ultrafiltrate of Human Plasma.

Bk = bovine bradykinin.
Uf = ultrafiltrate.

27", 35" etc indicate the latent period between administration of the dose to the bath and the onset of contraction.
RAT UTERUS - 30 ml. bath.

<table>
<thead>
<tr>
<th>Time</th>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>27&quot;</td>
<td>Bk</td>
<td>4x10^{-6} g</td>
</tr>
<tr>
<td>35&quot;</td>
<td>Uf Plasma</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>33&quot;</td>
<td>Bk</td>
<td>3x10^{-6} g</td>
</tr>
<tr>
<td>29&quot;</td>
<td>Bk</td>
<td>4x10^{-6} g</td>
</tr>
<tr>
<td>35&quot;</td>
<td>Substance P</td>
<td>10^{-5} g</td>
</tr>
<tr>
<td></td>
<td>Substance P</td>
<td>5x10^{-5} g</td>
</tr>
<tr>
<td></td>
<td>Bk</td>
<td>25x10^{-6} g</td>
</tr>
</tbody>
</table>
Ultrafiltrates of Burn Blister Fluid and of Plasma.

The pain produced by ultrafiltrates of burn blister fluid and of plasma resembles that of the parent materials.

\[ \text{ACH} = \text{acetylcholine chloride, g/ml.} \]
\[ \text{BBF} = \text{burn blister fluid.} \]

Subject: Clark
**Ultrafiltrate - Solubility in 85% Ethyl Alcohol**

(but NOT in Absolute Ethyl Alcohol).

Ultrafiltrate = $U_f$ = freeze dried ultrafiltrate of human plasma.

$B_k$ = bovine bradykinin preparation, g.

$R$ = residue of plasma after extraction by both absolute and 85% alcohol.

$Abs$ = extract of ultrafiltrate in absolute ethyl alcohol.

85% indicates extract in 85% ethyl alcohol.

The doses of ultrafiltrate residue and extractions in absolute and ethyl alcohol are equivalent to volumes of parent plasma.
RAT UTERUS

30 ml. bath

Uf plasma + EtOH

absolute 85%

Bk 3 x 10^-6

R Abs 85%

1 ml. 1 ml. 1 ml.
Figure 89.
Rat Uterus Contractions.

Action of Ethyl Alcohol and of Acetone on glass activated human Plasma - (a)
Action of Acetone (dry and 90%) on Ultrafiltrate of active human Plasma - (b)

A.E. = alcoholic extract,
p.a.p. = pre-active plasma,
gap = glass activated plasma,
Act.E. = acetone extract (acetone 95%)

Uf. = ultrafiltrate, freeze dried, reconstituted.
Act.'ppt' = 'residue' after extraction of freeze-dried ultrafiltrate in dry and 90% acetone,
E. = extract,
Act. = acetone

The doses given are equivalent to the volumes of parent plasmas from which these came.
Figure 90.
Ret Uterus Contractions.

Action of Acetone on Ultrafiltrate in the presence of added Protein.

Ultrafiltrate = ultrafiltrate of hydrocoele fluid, freeze dried.

(a). Extraction of ultrafiltrate alone:
P = 'precipitate'
E = extract in 95% acetone.
5-HT = 5-hydroxytryptamine creatinine sulphate.
90", 27" etc. indicates latent period before response.

(b). Extraction from ultrafiltrate in the presence of added protein (albumin).

The doses of precipitates and extracts are equivalent to the volumes of plasma from which they were derived.
Figure 91.
Rat Uterus Contractions.

Extraction of Ultrafiltrate Activity by Glacial Acetic Acid + Ether, in the presence of Protein.

Ultrafiltrate (Uf) is insoluble in diethyl ether itself. Even in the presence of protein it can be extracted to glacial acetic acid in ether.

Bk = bovine bradykinin, g.
Ppt. = residue after extraction.
E = extract.

The doses of precipitate and extract are equivalent to the volumes of parent plasma from which they were obtained.
RAT UTERUS

30 ml. bath

Uf plasma +
glacial acetic, ether, protein.

Bk $10^{-6}$
Ppt E Bk
$2 \times 10$

1 ml. 1 ml. 1 ml.
Figure 92.
Rat Uterus Contractions.

Ready digestion of Ultrafiltrate by
Chymotrypsin.

The two horns of a rat uterus were
separated and set up so as to give comparable
responses to a given dose of ultrafiltrate
of plasma (0.75 ml.). The responses of the
two horns to:

\[ Uf = \text{ultrafiltrate} \]
\[ Uf + ct \] = ultrafiltrate which had been
\[ Uf + T \] similarly incubated in the
presence of comparable
activities of chymotrypsin and
of trypsin, respectively,
are shown in the upper and lower records
respectively.
RAT UTERUS
30ml. bath

- Uf 0.75ml.
- Uf + Ct 1.5ml.
- Uf + T 1.5ml.
- Uf 0.75ml.

- Uf 0.75ml.
- Uf + T 1.5ml.
- Uf + Ct 1.5ml.
- Uf 0.38ml.
Inhibition of the Activation by Glass of pre-active human Plasma in the Presence of the Soya Bean Trypsin Inhibitor.

C = Control = pre-active human plasma which had been exposed to glass.

T = Test, i.e. pre-active plasma to which the trypsin inhibitor had been added (1mg/ml.) before transfer to glass.

Bk = bovine bradykinin.
RAT UTERUS

C  T  Bk

\[ t = 3' \quad 3' \quad 2\mu g. \]

0.1ml. 0.1ml.
Inhibition of the Decay of glass-activated human Plasma in the Presence of Disodium Versenate (disodium ethylenediamine tetra acetic acid).

The first administration is of pre-active plasma (- no exposure to glass - t = 0). The plasma was then transferred to glass, allowed to activate during seven minutes (7') and the same dose repeated. Normal glass-activation had taken place. At this time the sample was divided (\(\frac{1}{2}\)) into two aliquots, one to act as a control (c) (versene free) the other to have disodium versenate added to give a final concentration of \(10^{-3}\)g/ml. (V).

The figures 19', 23' etc. indicate total age of exposure of the plasma to glass min.

When tested at 40 min the control sample was inactive. By 46 min five times the original dose of control was also inactive. However the sample containing the versene was still active at 1\(\frac{1}{2}\) hr, the original dose, 0.1 ml. being employed.
Figure 95.

Rat Uterus Contractions.

1) t=10 sec  
2) t=1-2 sec  
3) t=0 sec  
Polythene Coils  
4) t=20 sec  
80°C
p.a.p. = pre-active human plasma

75\degree C for 15 sec indicates that the plasma was passed through fine bore coils of thin polythene immersed in a water bath of that temperature. Total passage time = 15 sec.

5-HT = 5 hydroxytryptamine creatinine sulphate.

Dihydroergotamine, D.H.E., to give a final concentration of $10^{-6}$g/ml. was added to the bath for 10 min before administration II. Its concentration in the bath was replenished after the washings which followed the subsequent administrations to the tissue.
Figure 96.

Rat Uterus Contractions.


Blood was taken from a normal subject and centrifuged to give platelet-rich and platelet-poor plasma.

<table>
<thead>
<tr>
<th>Administration Number</th>
<th>‘Treatment’</th>
<th>Nature of Plasma Platelet-rich = R Platelet-poor = P</th>
<th>Dose</th>
<th>Time of Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-i.e. p.a.p.</td>
<td>R</td>
<td>0.1ml</td>
<td>40″</td>
</tr>
<tr>
<td>2.</td>
<td>75°C for 15 sec</td>
<td>R</td>
<td>0.05ml</td>
<td>18″</td>
</tr>
<tr>
<td>3.</td>
<td>&quot; &quot;</td>
<td>R</td>
<td>0.025ml</td>
<td>25″</td>
</tr>
<tr>
<td>4.</td>
<td>-i.e. p.a.p.</td>
<td>P</td>
<td>0.1ml</td>
<td>18″</td>
</tr>
<tr>
<td>5.</td>
<td>3 kept for 12 min</td>
<td>R</td>
<td>0.025ml</td>
<td>18″</td>
</tr>
<tr>
<td>6.</td>
<td>75°C for 15 sec</td>
<td>P</td>
<td>0.05ml</td>
<td>18″</td>
</tr>
<tr>
<td>7.</td>
<td>3 kept for 25 min</td>
<td>R</td>
<td>0.025ml</td>
<td>30″</td>
</tr>
<tr>
<td>8.</td>
<td>5-HT</td>
<td>R</td>
<td>2.5 x 10^{-8}g.</td>
<td>17″</td>
</tr>
<tr>
<td>9.</td>
<td>exposed to glass for 3 min</td>
<td>P</td>
<td>0.025ml</td>
<td>18″</td>
</tr>
<tr>
<td>10.</td>
<td>3 kept for 40 min</td>
<td>R</td>
<td>0.025ml</td>
<td>23″</td>
</tr>
<tr>
<td>11.</td>
<td>5-HT</td>
<td>2.5 x 10^{-7}g.</td>
<td>30″</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>5-HT</td>
<td>5 x 10^{-8}g.</td>
<td>30″</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>exposed to glass for 3 min</td>
<td>P</td>
<td>0.025ml</td>
<td>12″</td>
</tr>
<tr>
<td>14.</td>
<td>3 kept for 80 min</td>
<td>R</td>
<td>0.05 + 0.2ml</td>
<td>-35″</td>
</tr>
<tr>
<td>15.</td>
<td>5-HT</td>
<td>0.025ml</td>
<td>16″</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>exposed to glass for 3 min</td>
<td>5-HT</td>
<td>2.5 x 10^{-8}g.</td>
<td>40″</td>
</tr>
</tbody>
</table>

continued on previous page
Figure 97.

Rat Uterus Contractions.

Extract in 95% Acetone from heat activated platelet-rich human Plasma.

Ppt = precipitate, 'reconstituted'
E = extract
5-HT = 5-Hydroxytryptamine creatinine sulphate.

The volumes of precipitate and extract administered are equivalent to those of the parent plasma.

The dose of extract which gives the marked response is one fifth of that of precipitate which gives a medium response only.
Figure 98.

Rat Uterus Contractions.

Unstable oxytocic Activity from heated human Plasma.

Upper record:

A.E. = Alcoholic extract from a glass activated plasma

1, 4, 8 min indicates duration of incubation of the plasma in polythene pot in a water bath at 60°C.

Lower Record:

P.A.F. = pre-active plasma

15 sec, 1 min, etc., indicates duration of heating

'tested at 2.5 min' indicates time interval between collection of the fluid from the heater and administration to the bath.
RAT UTERUS
5 ml. bath

Plasma — bath 60° C

2 min

AE 1 min  4 min  8 min  A.E.
0.1  0.1  0.1  0.1
ml.  ml.  ml.  ml.

tested at once.

RAT UTERUS
5 ml. bath

Plasma — bath 55° C

PAP  15 sec  1 min  12 min
0.2  0.1  0.1  0.1
ml.  ml.  ml.  ml.

tested at 2.5 min
Figure 99.

Rat Uterus Contractions.

Action of Atropine on aqueous Extract of Rat Skin.

The extract, E, was made by chopping the skin in a volume, ml., of R.L. solution equal to the wet weight of the tissue, g.

$\text{ACH} = \text{acetylcholine chloride.}$

$\text{Atropine} = \text{atropine sulphate.}$ The concentration given is the final concentration in the bath.

It will be seen that though the atropine blocked the response to ten equivalents of ACh it did not do so to two equivalents of extract. On washing out, the sensitivity of the tissue to one equivalent of extract returned immediately, whereas that to ACh remained blocked.
Figure 100 (a).

Rat Uterus Contractions.

Action of Dihydroergotamine on Contractions induced by Rat Skin Extract.

\[
\begin{align*}
5\text{-}HT & = 5\text{-hydroxytryptamine creatinine sulphate.} \\
R\text{.Sk.E.} & = \text{rat skin extract} \\
D\text{.H.E.} & = \text{dihydroergotamine. The concentration is the final concentration in the bath.}
\end{align*}
\]

Though the action of 5-HT was blocked ten fold or more by the D.H.E., the response to skin extract was unaffected.
Figure 100(b).

Rat Uterus Contractions.

Action of Dihydroergotamine on Contractions induced by Ultrafiltrate of Rat Skin Extract.

5-HT = 5-hydroxytryptamine creatinine sulphate.
Uf = ultrafiltrate of rat skin extract.
D.H.E. = dihydroergotamine. The concentration indicated is the final concentration in the bath.

Here in the face of about a two-hundred fold blockade of 5-HT response by D.H.E. the action of a single dose of Uf of extract was unaffected.
Figure 101.

Rat Uterus Contractions.

Rat Skin Extract kept at 0°C and 35°C

E = extract
Bk = bovine bradykinin
E35 = extract kept at 35°C
EO = extract kept at 0°C

12 min, 32 min, etc., indicate the times of keeping of the extract (E35) at 35°C. The durations of keeping of EO samples at 0°C are intermediate.

48", 42", etc., indicate the latent period between administration of solutions to the bath and the onset of the contraction by the tissue.

By 1 - 2 hr the response to E35 was greatly reduced whereas that to EO was still as great as ever.

Notice the difference in configuration of the E, E35 (early responses), and EO responses and that of the typical effect of Bk. I have found such differences to be significant and in fact the active principle of skin extract is NOT bradykinin.
Figure 102.

Rat Uterus Contractions.

Digestion of Ultrafiltrate of Rat Skin Extract by human Plasma.

Bk = bovine bradykinin.
P = human plasma.
Uf = Ultrafiltrate of aqueous extract of rat skin.

20°C, 35°C, etc., indicate the temperatures of incubation for the durations stated.

At 35°C were incubated:—

(i) Uf alone
(ii) P alone
(iii) Uf + P
(iv) Uf + R.L.

At R.T., 20°C, were incubated:—

(i) Uf
(ii) R.L.

Response 4, shows that 0.4ml. Uf gives a good response, and 5 that 0.1ml of the 'decayed' plasma fails to give a response.

These, freshly mixed gave a good response, (response 6), as did the same amount of Uf mixed with R.L. and incubated for a few minutes (response 7)
Figure 103.

Rat Uterus Contraction.

Action of Acetone on Rat Skin Extract.

5-HT = 5-hydroxytryptamine creatinine sulphate.
ppt = 'precipitate'.
R.Sk.E = rat skin extract.

Equivalent doses of precipitate
(reconstituted) and of extract were given. The
activity was acetone soluble.
Figure 104.

Rat Uterus Contractions.

Action of Acetone on freeze dried Ultrafiltrate of Rat Skin Extract and of ditto in the presence of added Protein.

Act. = acetone
E. = extract
Ppt. = precipitate
Pr. = protein

The doses are equivalent to volumes of the parent extract (and ultrafiltrate).

The figure shows that the ultrafiltrate activity is acetone soluble. When protein (albumen) was added before extraction almost all the activity once more appeared in the extract. (Compare Fig. 90).
RAT UTERUS

5 ml. bath.

Act. E. 0.25 ml.
Act. Ppt. 0.5 ml.
Act. + Pr. E. 0.25 ml.
Figure 105.

Pain Records.

Heat Burn of the Forearm.

The initial spike of each record indicates the pain due to application of a hot rod to the forearm during 3 sec. The temperatures of the rods were 65 and 70°C respectively.

On removal of the hot rod the pain subsided to zero (or almost so) very rapidly. Then there followed a blank period during which no pain was felt (indicated 27", 21"). Pain then arose, slowly mounted, reached a well-maintained peak and then, after several minutes, gradually subsided.
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[Text content not visible]
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PUBLICATIONS.
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The following publications by the author contain information relevant to the subject of the thesis:

   
   
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2. Pain-producing substances in blister fluid and in serum.
   
   
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