(3) The two-point attachment theory is complicated on the grounds of configurational entropy, whereas the attachment to a variable area of the membrane's surface similar to the areas determined by three I⁻ (see Fig. 2), together with a better locking of the membrane, allows more randomization in the attachment of the *bis*-cationic and mono-cationic chains.

Since acetylcholine is a mono-cation one would expect that it travels during the synaptic transmission with the quaternary cationic group oriented towards the receptor membrane and the esteratic end towards the emitting membrane. Thus, the hypothesis of Wilson[5] that acetylcholine is hydrolysed after absorption by the anionic and esteratic sites of cholinesterase present on the surface of the receptor membrane, would be acceptable if the positions of the sites in space is such that the hydrolysis takes place without fundamentally changing the travel orientation of acetylcholine. The last hypothesis does not contradict the blocking mechanism of the methonium chains.

It is of interest that *n*-amyl-trimethyl ammonium, a mono-cation with a similar shape to acetylcholine, possesses the maximum acetylcholine-like properties within the alkyl-trimethyl ammonium series[19].

If the same rules apply to the forces acting in the solid state as to those in the absorption state, these rules would be particularly applicable between the blocking agents and the receptor sites, because the ionic bonds are not strong enough to allow for the dielectric constant of water between the membrane's surface and the non-hydrolysable blocking agents. If the chains were only partially ionized they would be able, under the synaptic difference of potential, to orient themselves with the N^+ charges towards the receptor sites and the halide anionic charge towards the emitter membrane, thus permitting the attachment to the anionic layer of new cations, such as acetylcholine and methonium *bis*-cations, which would block nervous transmission.

The assumption that the same sort of rules apply in the solid state as in the absorption state is supported by the fact[6] that the blocking activity of the less bulky quaternary groups (trimethyl ammonium) is a function of the chain's length, whereas more bulky quaternary *bis*-cations such as $(C_2H_5)_3N^+—R—N^+—(C_2H_5)_3$ do not allow the solid state forces to act and they block the nervous transmission by their bulky presence independently of the chain length of *R*.

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IODINE BINDING BY PROTEINS IN CANINE GASTRIC MUCUS

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RECENT demonstrations of the presence of serum proteins in normal anacid gastric juice, or in gastric juice collected with buffering *in situ*, have prompted quantitative estimates of their occurrence. Wetterfors *et al.*[1] estimated that in human beings about 1·3 gm. of albumin per 24 hr. is eliminated by the stomach. Horowitz and Hollander[2] found the albumin content of acetylcholine (ACh)-mucus from dogs ranged from 4 to 10·2 mgm./ml. of mucus. Both groups of investigators used serum albumin labelled with iodine-131 and based their calculations on the fraction of iodine-131 in the gastric secretion that was protein bound—about 10 per cent in the human gastric juice, and 25–75 per cent in the canine ACh-mucus. (For convenience, such data will be referred to as 'per cent binding'.) Since some of the non-protein-bound radioactive substances may be inorganic iodide, and since it has been demonstrated that this iodide may be bound *in vivo* by endogenous protein other than that of the thyroid—at least in the salivary glands[3] and in the mammary glands[4]—we undertook an exploratory investigation of the possible occurrence of such binding in gastric mucous secretion.

Carrier-free sodium iodide (labelled with iodine-131, 2–5 μc./kgm. body-weight, was administered intravenously to 5 Heidenhain pouch dogs in which antrectomy had been performed. 16 hr. after labelled sodium iodide administration, ACh-stimulated mucus was collected over an 8-hr. period. Blood samples were obtained at the beginning and end of the collection of mucus. After homogenization of the mucus, its protein-bound content labelled with

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iodine-131 was measured in separate aliquots by three methods: alkaline zinc sulphate precipitation, dialysis for 36 hr. against running water, and passage through an ion-exchange resin column. Serum protein-bound iodine-131 was separated by alkaline zinc sulphate or ion-exchange resin. Radioactivity counting was performed in a well-type scintillation counter. Similar results were obtained by the three analytical methods. Moreover, dialysis, with and without subsequent $ZnSO_4$ precipitation, gave essentially the same radioactivity of the sample, suggesting that almost complete separation of protein-bound iodine-131 was achieved by dialysis alone.

Paper electrophoresis of the mucus specimens was performed in veronal buffer (pH 8·6, $\Gamma/2$ = 0·075). The paper strip was divided into 1-cm. segments, each of which was counted in the well counter.

The results are presented in Table 1. It will be observed that in one of the dogs (No. 388), in three separate experiments, the protein-bound iodine-131 content of the mucus ranged from 17·0 per cent to 34·1 per cent (mean = 21·9 per cent regardless of method) of the total radioactivity of this mucus specimen. Moreover, when this dog was pretreated with Lugol's solution for 4 days, the protein-bound iodine-131 was reduced to 2–4 per cent of total iodine-131, possibly indicating that binding sites were being saturated by this pretreatment. In the other four dogs, the per cent binding was lower, averaging 4·9 per cent of the total radioactivity by the zinc sulphate method, 2·4 per cent by dialysis, and 2·8 per cent by ion-exchange resin. Serum protein-bound iodine-131 values ranged from 0·9 per cent to 6·6 per cent (mean = 3·0 per cent).

Table 1. Per Cent Binding of Inorganic Iodide by Proteins in Canine Gastric Mucus, determined by Three Separate Methods

Dog specimen	$Na^{131}I$ administered (μc./kgm.)	Initial blood $PB^{131}I$ (μc./kgm.)	Final blood $PB^{131}I$ (μc./kgm.)	$PB^{131}I$ as per cent of total ^{131}I in ACh-mucus		
				$ZnSO_4$	Dialysis	Resin exchange
388–*A*	5·0	3·3	3·7	21·6	18·4	17·0
388–*B*	2·5	2·7	2·9	19·6	17·4	25·0
388–*C*	3·8	1·2	2·1		34·1	
388–*D**	3·4	0·7	0·9	4·0	2·1	
379–*A*	2·5	2·5	5·2	3·4	1·4	1·8
379–*B*	5·0	3·2	3·3	7·4	1·6	1·1
392–*A*	2·0	1·7	4·1	5·6	4·0	2·5
392–*B*	4·0	0·9	2·2		1·0	
394–*A*	3·0	5·5	6·1	5·2	4·7	
363–*A*	2·0	2·6	6·6	2·6	1·3	1·1

* After pre-treatment with Lugol's solution.

Paper electrophoresis of the homogenized specimen of mucus was performed with four of the specimens. Material from dog No. 388, the animal with the highest degree of binding, gave an electrophorogram in which the protein-bound iodine-131 was distributed throughout the pattern, but the greatest amounts remained at the origin (Fig. 1). Paper electrophoresis (the same duration) of the fluid fraction of this mucus, after separation from the gel by centrifugation, revealed less radioactivity at the origin and more moving with the anodic fractions (Fig. 2). The high level of protein-bound iodine-131 at the origin in the unseparated homogenate was most probably the result of the former contained in the immobile gel aggregate. Paper electrophorograms of specimens with minimal binding (for example, one from dog No. 392, for which the binding was only about 4 per cent of the total radioactivity) manifested barely detectable radioactivity. However, agar electrophoresis and autoradiography, which utilized a larger sample than did paper electrophoresis, indicated that the labelled protein remained immediately adjacent to the origin.

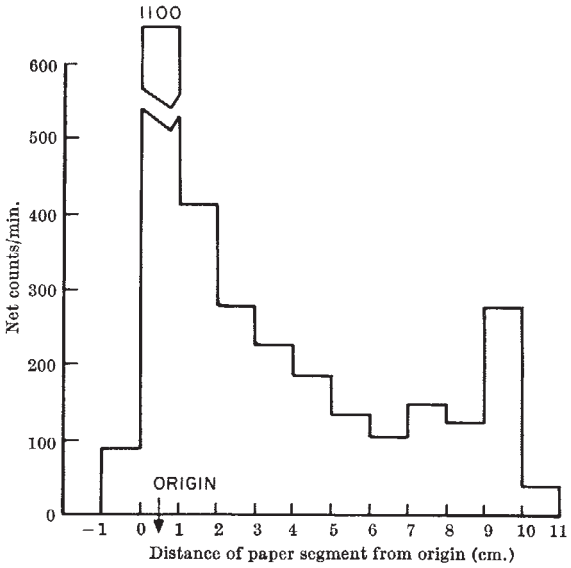

Fig. 1. Distribution of radioactivity in paper electrophorograms of homogenized acetylcholine mucus (veronal buffer pH 8·6, $\Gamma/2$ = 0·075). Paper was divided into 1-cm. segments and counted in a well-type scintillation counter. (Data from 6 strips pooled for counting)

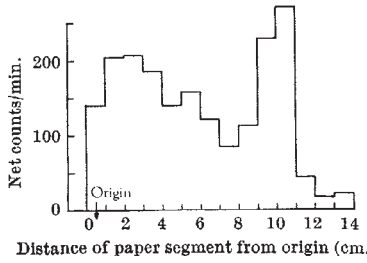

Fig. 2. Paper electrophoresis on supernate from homogenized, dialysed, and centrifuged acetylcholine mucus (veronal buffer pH 8·6, $\Gamma/2$ = 0·075). Paper was divided into 1-cm. segments and counted in a well-type scintillation counter. (Data from 6 strips pooled for counting)

It appears, therefore, that *in vivo* binding of inorganic iodide to proteins occurs in the gastric secretion as well as in the salivary and mammary glands, at least in some dogs, and can lead to uncertainties in the quantitative estimation of serum proteins in this secretion. Errors from this source may be particularly pronounced if the ratio of:

$$\frac{\text{total iodine-131 counts}}{\text{protein-bound iodine-131 counts}}$$

is high. The magnitude of error which may be encountered can be demonstrated by the following example. If 4·5 nc. protein-bound iodine-131/ml. mucus, and 32·5 nc. total iodine-131/ml. mucus, are encountered, and if a binding by gastric protein of 10 per cent occurred, then approximately 2·8 of the 4·5 nc. of protein-bound iodine-131 in the mucus may derive from this binding—giving an error of about 62 per cent in the quantitation. Pretreatment with Lugol's solution may reduce the order of binding, as indicated by the experiment with specimen No. 388-*D*, thus reducing the degree of error but not eliminating it completely. It must be noted that the quantitative uncertainties presented by these

observations in no way invalidate our previous findings regarding the presence of serum proteins in non-acid mucinous secretions. This statement is supported by the finding of precipitin bands in the albumin and globulin regions on immunoelectrophoresis of the specimens utilized in this investigation.

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RELATIONS BETWEEN CHANGES IN MEMBRANE PERMEABILITY AND THE CLIMACTERIC IN BANANA AND AVOCADO

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THE occurrence of marked alterations in membrane permeability and cellular dissociation (separation of cells at the middle lamella) was observed attending senescence of fruit and leaf tissues[1,2]. These phenomena were demonstrated in leaf section of *Rhoeo discolor* and *Mesembryanthemum* sp., in abscission zones of *Coleus blumei* (also *Fuchsia* sp. and *Cestrum nocturnum*, unpublished), and in the endocarp of Kentucky Wonder pole beans. In each of these tissues senescence could be delayed considerably by the addition of an auxin, that is, indolyl-3-acetic acid or α-naphthalene acetic acid. Indications of permeability changes were liquid-logging of intercellular spaces, exudation from tissue surfaces and a marked increase in both the rate of plasmolysis and leakage of endogenous solutes.

Further investigations on bean endocarp demonstrated clearly that senescence was attended by a progressive increase in the free space of the tissue from 10 to 70 per cent over 6 days[3,4].

Microscopic examination of sections of fleshy fruits (pomes, drupes and berries) showed the occurrence of liquid-logging of intercellular spaces and cellular dissociation during the ripening process[2]. Similar results were observed during incubation of slices of these fruit tissues. The evidence of changes in permeability during the ripening of climacteric fruits suggested that alterations in membrane properties may be causative of the climacteric.

In this article the results of studies on relations between permeability changes and the climacteric rise in respiration in banana and avocado are reported. Attempts to determine changes in permeability with time by the method of Briggs and Robertson[5] for measuring free space were unsuccessful, owing to the marked leakage of solutes (sugars) in bananas during ripening. Attempts were made also to measure the rate of leakage by recording loss of weight while the tissue was suspended in distilled water by a human hair from the beam of an analytical balance. The accuracy of this method was hampered by the occurrence of variable uptake of water, which often masked weight loss due to leakage until the latter process becomes predominant. A number of trials using these methods, however, indicated that the onset of marked leakage attended the beginning of the climacteric rise—and that, paralleling the respiratory rise, there was a progressive increase in the rate of leakage.

The data reported here were obtained from measurements of the dry weight of diffusates. This method, described following, proved to be consistently reliable for determining changes in the rate of leakage of solutes.

Bananas[6] were maintained at 22·5° C and high relative humidity for both respiratory and permeability studies. Respiratory measurements were based on evolution of carbon dioxide, measured with a Blackman model 15*A* infra-red carbon dioxide analyser, coupled with an Angus–Esterline recorder. The analyser was calibrated with a standardized gas mixture of nitrogen with 515 p.p.m. carbon dioxide. A constant flow of compressed air was maintained with a micrometer needle valve, and the flow-rate measured with a calibrated Brooks Rotameter. The air was dried over silica gel and passed through 'Ascarite' to remove carbon dioxide, and then humidified before entering the fruit chamber.

In these studies, either bananas from the hand from which fruits were removed for respiratory studies were used, or the same bananas were used for both respiratory and leakage studies. Cross-sections of banana tissue (rind-free) were removed daily, aspirated for 0·5 h at 47 mm mercury in distilled water, and then brushed gently to remove air bubbles and any loose fragments of tissue. The sections were suspended in a wire basket in 400 ml. of fresh distilled water for 3 h. The ambient solution was filtered through Whatman No. 1 paper, concentrated under vacuum, and the dry weight of the diffusate determined.

An increase in the rate of leakage attended the onset of the climacteric rise in respiration, and further, there occurred a progressive increase in the rate of leakage attending the respiratory rise (Fig. 1). The same results were obtained in two other experiments conducted similarly. Experiments conducted with and without aspiration established that the aspiration had no adverse effects. Likewise, it was determined that there was no difference in leakage, with and without added calcium in the ambient solution.

Experiments with Haas and Fuerte variety avocados showed a similar correlation between increased leakage and the climacteric rise. In these trials, however, the method involving the measurement of loss of weight while the tissue was suspended in water from the beam of an analytical balance was used. Notwithstanding the aforementioned limitations of this method, it was clear that an increased rate of leakage attended the respiratory rise.

The following interpretation is suggested for the correlation observed between the progressive increase